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Cáncer y diabetes: influencia del estado pro-inflamatorio diabético en las características del cáncer de colon

Memoria presentada para la obtención del grado de Doctor en Medicina

por

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A todos los residentes de mi especialidad,
para que no olviden la importancia
de la investigación rigurosa en Oncología,
a cualquier edad y en cualquier momento.

Contenido

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ABREVIATURAS

DMT2 Diabetes mellitus tipo 2

DM Diabetes mellitus

CCR Cáncer colorrectal

ADA American Diabetes Association

DMT1 Diabetes mellitus tipo 1

GLP-1 Glucagon like peptide-1

TGF-ß Factor de crecimiento transformante ß

EMT Transición epitelio-mesenquimal

MMR Mistmach repair system
Sistema reparador de desequilibrio

MSI Inestabildad de microsatélites

TCGA The Cancer Genome Atlas Network

MAPK Mitogen activated protein kinase
Proteínas quinasas activadas por mitógenos

GSK-3ß Quinasa glucógeno-sintasa 3-ß

VEGF Vascular endothelial growth factor
Factor de crecimiento endotelial

SLE Supervivencia libre de enfermedad

SLP Supervivencia libre de progresión

SG Supervivencia global

EGFR Epidermal growth factor receptor
Receptor del factor de crecimiento epidérmico

RIE Ratios de incidencia estandarizada

NFD Nefropatía diabética

IMC Índice de masa corporal

OR Odds Ratio

IC Intervalo de confianza

RR Riesgo relativo

HR Hazard Ratio

HbA1c Hemoglobina glicosilada

PFGs Productos finales de la glicolisis

IGF-1 Insulin-like growth factor
Factor de crecimiento insulina-like

PI3K Phosphatidyl-inositol-3-kinase
Fosfatidil inositol 3-quinasa

PAK-1 Activated protein kinase-1
Proteína quinasa-1 activada

ChREBP Carbohydrate response element-binding protein
Proteína de unión al elemento de respuesta a carbohidratos

NF-IB NF-κB- Factor de transcripción
Nuclear factor-kappa B

ATP Adenosina trifosfato

Glut-1 Glucotransporter-1

ECR Ensayos clínicos randomizados

ARVD Activadores del receptor de la vitamina D

S1P Esfingosina-1-fosfatasa

SEPT9 Septina 9

mRNA RNA mensajero

miRNA MicroRNAs

GWAS Genome-wide association studies

SNP Polimorfismo de nucleótido sencillo
Single Nucleotide Polymorphism

SEER Surveillance, Epidemiology and End Results

CC Cáncer de colon

ECOG Eastern Cooperative Oncology Group

CEA Antígeno carcinoembrionario

STZ Estreptozotocina

STZ-D Diabetes inducida por estreptozotocina

RESUMEN

La diabetes mellitus tipo 2 y el cáncer son enfermedades de epidémicas proporciones a nivel mundial. Las muertes atribuidas a estas dos enfermedades se han incrementado entre un 90% y un 57% respectivamente a lo largo de los últimos 20 años. El riesgo de cáncer colorrectal se estima un 27% más alto en pacientes con diabetes tipo 2 que en controles no diabéticos, aunque existen muchos factores de confusión en los estudios publicados. A nivel mundial, no existe una correlación clara entre la prevalencia de la diabetes mellitus tipo 2 y la incidencia del cáncer colorrectal. Las estimaciones de esta asociación se han modificado a lo largo de los años, sugiriendo el impacto de factores ambientales coexistentes.

El cáncer colorrectal comparte algunas vías celulares y moleculares implicadas en el daño producido en los órganos diana de la diabetes. Estas vías incluyen daño a las células epiteliales, activación de la inflamación y vías con implicación del factor de crecimiento epidérmico o de Wnt/ß-catenina, entre otros. Además, el tratamiento para la diabetes puede impactar en la aparición o evolución del cáncer colorrectal: la insulina podría estar asociada con un aumento de la incidencia de cáncer colorrectal mientras que a la metformina se le asocia un efecto protector.

Revisada esta evidencia, existen suficientes estudios epidemiológicos que analizan el posible mayor riesgo de padecer cáncer colorrectal en pacientes diabéticos pero no hay estudios que indiquen si existen diferencias en las características de estos tumores, una vez que la enfermedad está establecida. Esta tesis estudia las posibles diferencias en el cáncer colorrectal atribuibles al microambiente que condiciona la diabetes mellitus. Con este objetivo, se ha analizado una base de datos de pacientes con cáncer colorrectal, diabéticos y no diabéticos, y se ha desarrollado un modelo animal en el que se indujeron las dos enfermedades, estudiando las diferencias del cáncer en ratones con y sin diabetes, tanto a nivel histológico como molecular. Según la hipótesis de trabajo de esta tesis, conocido el ambiente pro-inflamatorio que rodea a la diabetes y que es responsable de diversas alteraciones en los órganos diana, los individuos que sufren diabetes deberían de ser más proclives a sufrir tumores con características diferentes, presuponiéndose mayor agresividad a nivel clínico, histológico o molecular, así como diferente respuesta a los tratamientos estándar.

1. INTRODUCCION

La diabetes mellitus tipo 2 (DMT2) y el cáncer son enfermedades de epidémicas proporciones a nivel mundial [1]. Se estima que en España un 14% de la población padece diabetes mellitus (DM) asociado con daño renal, enfermedad ósea y mortalidad temprana por enfermedad cardiovascular [2]. El cáncer es la segunda causa de muerte en España, siendo la primera causa en hombres y la segunda en mujeres [3]. Numerosos estudios epidemiológicos han identificado asociaciones entre estas dos enfermedades, la DM y los distintos tipos de cáncer. El riesgo de cáncer de mama, colon, recto, vejiga, linfoma no Hodgkin y riñón es un 20-40% más alto en la población con diabetes tipo II [4,5].

Cáncer y DM comparten factores de riesgo: sexo, estilos de vida, sobrepeso y hábitos dietéticos pobres. También a nivel molecular, un preciso conocimiento de las complejas asociaciones e interacciones entre estas enfermedades sería de gran importancia para su adecuada prevención y tratamiento. Posibles mecanismos comunes postulados para un enlace biológico entre DM y cáncer incluyen hiperinsulinemia, hiperglicemia e inflamación [6]. Datos recientes han difundido el concepto de que la inflamación es un mecanismo crítico de la diabetes y de la iniciación y progresión tumoral. Se estima que un 15% de los tumores se asocian a inflamación crónica. Ejemplos de este dato es el cáncer de colon asociado a las enfermedades inflamatorias intestinales crónicas y el hepatocarcinoma en hepatopatía crónica [7]. Por otro lado, la hiperglicemia e hiperlipidemia, comunes en pacientes con diabetes, activan diferentes vías proinflamatorias, bien directamente, bien vía transcripción génica que inducen estrés oxidativo [6].

Según los datos de la Global Burden of Disease, entre 1990 y 2013 la mortalidad debida a la DM aumentó un 90%, hasta 1.299.000 muertes anuales. En 2013, la Federación Internacional de Diabetes estimó a nivel mundial en 382 millones las personas con DM, siendo la cifra estimada para 2035 de 592 millones (www.diabetesatlas.org/). El cáncer colorrectal (CCR) figura entre las causas top de muerte por cáncer. Entre 1990 y 2013, la muertes relacionadas con CCR aumentaron un 57% hasta 771.000. En los Estados Unidos el CCR es la segunda causa de muerte por cáncer en hombre y mujeres combinado [8]. El CCR es la causa más frecuente de muerte por cáncer en varones (746000 casos anuales, 10% del total) y la segunda en mujeres (614000 casos, 9% del total) a nivel mundial. Tras el consenso alcanzado en 2010, la relación entre DM y cáncer ha sido reconocida en las guías de la American Diabetes Association (ADA) [9]. Sin embargo, actualmente esta aseveración ha tenido poco impacto en la asistencia clínica ya que no se realizan test de diagnóstico específicos ni se aplican tratamientos preventivos apoyados por recomendaciones clínicas. Por otro lado, analizando datos a nivel mundial, la prevalencia de la DM y la incidencia del CCR no se correlacionan. Este hallazgo sugiere que podrían existir factores específicos de cada país que jugarían algún papel en la asociación descrita entre DM y CCR. En este sentido, la incidencia del CCR varía con un factor multiplicativo de más de 10 a lo largo de los distintos países, siendo la frecuencia más alta estimada la de Corea (frecuencia estandarizada por edad de 45 por 100.000), y países como Australia (38 por 100.000), Irlanda (35) y países del oeste africano (por ejemplo Camerún, con 3,3) las frecuencias más bajas (http://globocan.iarc.fr). Por el contrario, la prevalencia más elevada de DM se encuentra en Egipto y en lo Emiratos Árabes Unidos (20.000 y 19.300 por 100.000), mientras que Australia (5.100), Irlanda (4.400) y algunos países del oeste de África muestran las prevalencias más bajas (www.diabetesatlas.org/) Un mejor entendimiento de los factores que subyacen bajo estas diferencias regionales podría darnos algunas claves de la posible relación entre la DM y el CCR.

1.1. Diabetes mellitus

La DM se caracteriza por un estado de hiperglucemia resultante de un defecto en la secreción de insulina, en su mecanismo de acción, o en ambos. La hiperglucemia crónica está asociada al daño, disfunción y fracaso a largo plazo de algunos órganos como riñones, corazón, nervios periféricos, retina y vasos sanguíneos [10]. En la diabetes tipo I (DMT1, 5-10% de

los casos de DM), la destrucción autoinmune de las células ß del páncreas producen una deficiencia absoluta de insulina. La DMT1 es el resultado de un proceso autoinmune crónico que normalmente subyace en los años previos al debut de la enfermedad, que suele hacerlo de forma brusca en forma de hiperglucemia y cetosis. Los principales genes asociados a esta herencia están localizados en el cromosoma 6, en genes implicados en el reconocimiento de moléculas que conforman el sistema de histocompatibilidad HLA [11].Dado que el mecanismo fisiopatológico de este tipo de diabetes difiere completamente del tipo 2, que es el subtipo que podría estar más relacionado con un ambiente inflamatorio y establecer mecanismos comunes con otras enfermedades como el cáncer, no es objeto de esta tesis ahondar más en sus características.

La DMT2 se define por la resistencia a la insulina y la deficiencia de esta hormona. Tras la ingestión de glucosa, el balance homeostático de la misma depende de tres procesos que deben de ocurrir de forma coordinada: (1) la secreción de insulina; (2) la estimulación de la absorción de glucosa por el hígado, el intestino y tejidos periféricos en respuesta a la hiperinsulinemia o a la hiperglucemia; (3) la supresión de la producción hepática de glucosa. La DMT2 se caracteriza por 4 anormalidades metabólicas: obesidad, disminución/resistencia a la acción de la insulina, disfunción de la secreción de la misma y aumento de producción de glucosa endógena. Parece que al menos 3 de estas anormalidades condicionan el inicio de la DM, aunque se desconoce la secuencia en ausencia de estudios longitudinales. Estudios transversales y prospectivos demuestran que la obesidad y la resistencia a la insulina podrían ser factores predictores de DM años antes del inicio de la enfermedad, aunque la información detallada sobre el curso de la enfermedad es variable entre individuos y aún se desconoce [12].

Los casos de DMT2 son con frecuencia pacientes obesos y de edad avanzada en el momento del inicio de su proceso comparados con los casos de DMT1 [13]. Una herencia genética de defecto en la células ß o en la maquinaria de señalización de la insulina causan también DM, aunque no se conocen con detalle los defectos genéticos específicos como ocurre en la DMT1. La base genética de algunas formas monogénicas de DMT2 como son el síndrome de Wolfram, la asociación de diabetes y sordera debido a un defecto genético mitocondrial o los síndromes MODY (maturity onset diabetes of youth), sólo justifican una pequeña muestra de todos los casos de DMT2 [10].

Las terapias para la DM incrementan la disponibilidad de la insulina, mejoran la sensibilidad a esta hormona, disminuyen la síntesis de glucosa, retrasan la absorción intestinal de los carbohidratos y aumentan la secreción urinaria de glucosa. La tabla 1 muestra los principales agentes utilizados en el tratamiento de la DMT2, sus mecanismos de acción y su acción sobre el peso. Las terapias basadas en el Glucagon-like peptide-1 (GLP-1) imitan los efectos de las incretinas. Estas moléculas son hormonas intestinales que aparecen en respuesta a una ingestión de alimentos que a su vez estimulan la secreción de insulina y limitan la liberación del glucagón[14-16]. El agente farmacológico más utilizado como primera opción en la DMT2 es la metformina, droga que disminuye la producción de glucosa inhibiendo la glicerolfosfato deshidrogenasa mitocondrial [17]. Si no se alcanza un adecuado control de las cifras de glucosa en los 3-6 primeros meses, se administra un segundo fármaco, agonista del receptor de GLP-1, o se añade insulina [18,19]. El desarrollo de insuficiencia renal asociada a DM puede limitar el uso de metformina ya que ésta puede favorecer la aparición de acidosis láctica. En ese caso, la enfermedad renal es un factor de confusión asociado a la mortalidad por cualquier causa [20].

	Agente	Ruta	Diana Molecular	Impacto en la homeostasis de la glucosa y de la insulina	Impacto en el peso	Impacto en el cáncer colorrectal
Activan el receptor de insulina/ Aumentan la secreción de insulina	Insulina	Parenteral	Activa el receptor de insulina	Aumenta la insulina	Aumenta	Aumento/neutral/ descenso
	Sulfonilureas	Oral	Inhibe el receptor para sulfonilureas/ modula la actividad de los canales de calcio dependientes de ATP en las células ß pancreáticas	Aumenta la secreción de insulina	Aumenta	Neutral
	Terapias basadas en GLP-1: inhibidores de DPP-4	Oral	Inhibe DPP-4 y disminuye la degradación de incretinas	Estimula la secreción de insulina dependiente de glucosa, enlentece el vaciado gástrico, reduce en glucagón postprandial	Neutral	Neutral
	Terapias basadas en GLP-1: agonista del receptor GLP-1	Parenteral	Activa el receptor GLP-1		Neutral/ disminuye	Neutral/disminuye
Sensibilizan para la acción de la insulina	Tiazolidinas	Oral	Activa el receptor gamma activado por el factor proliferador de peroxisomas (PPAR-)	Sensibiliza para la acción de la insulina: aumenta la utilización de la glucosa y disminuye la producción de glucosa	Neutral	Neutral/disminuye
Disminuye la síntesis o la absorción de glucosa	Metformina	Oral	Inhibe la enzima glicerolfosfato deshidrogenasa	Disminuye la producción de glucosa	Disminuye	Disminuye
	Inhibidores de la a-glicosidasa	Oral	Inhibe la a-glicosidasa	Disminuye la absorción de glucosa intestinal	Disminuye	Neutral/disminuye
	Inhibidor de SGLT2	Oral	Inhibe el transportador 2 de glucosa ligado a sodio	Aumenta la pérdida de glucosa urinaria	Disminuye	Neutral

Tabla 1. Tratamiento de la diabetes tipo II con sus mecanismos de acción, su impacto en el peso y su posible relación con el CCR. GLP-1: Péptido glucagón-like-1; DPP-4: Dipeptidil peptidasa-4; SGLT2: Transportadores de glucosa ligados a sodio.

1.2. Cáncer colorrectal

1.2.1. Generalidades:

El CCR se origina en el epitelio del colon. Se han descrito distintos patrones dependiendo de los factores de riesgo ambientales o genéticos [21]. El cáncer de colon hereditario incluye el síndrome de Lynch o cáncer colorrectal hereditario no polipósico y síndromes polipósicos como la poliposis adenomatosis clásica o atenuada. Este tipo familiar (25% de los casos) está asociado con historia familiar de CCR o adenomas de gran tamaño, en ausencia de herencia mendeliana [22]. La enfermedad esporádica (70% de los casos de CCR) ocurre la mayoría de los casos por encima de los 50 años de edad y su incidencia aumenta con la edad, posiblemente como resultado de factores ambientales y dietéticos. El CCR esporádico muestra alteración de vías de señalización críticas tales como la inactivación de TP53, BRAF, mutaciones en PI3CA, inactivación de APC, KRAS, factor de crecimiento transformante ß (TGF-ß), mutaciones en CTNNB, disregulación de los genes de transición epitelio-mesenquimal (EMT), activación de la señal de Wnt y amplificación de MYC, entre otros. Estas diferencias moleculares condicionan las características fenotípicas de cada CCR. La tabla 2 muestra las principales alteraciones genéticas implicadas en el tumor [23,24].

Cáncer Colorrectal	Mutación	Herencia	Impacto de la DM en la expression génica	Referencia
Poliposis adenomatosa familiar	Línea germinal del gen adenomatous polyposis coli (APC)	Autosómica dominante	Sobre-expresión de APC	[25,26]
Poliposis asociada a MYH	Línea germinal de MYH	Autosómica recesiva	MYH sin alterar	[25,26]
Síndrome de Peutz-Jeghers	Línea germinal de la quinasa serina treonina (STK11)	Autosómica dominante	Sobre-expresión de STK11	[27]
Cáncer hereditario familiar no polipósico (Síndrome de Lynch)	Línea germinal de genes MLH1, MSH2, MSH6, o PMS2	Autosómica dominante	MLH1, PMS2 sin alterar Sobre-expresión de MSH2, MSH6	[28]
Inestabilidad cromosómica	Acumulación adquirida numérica (aneuploidia) o anormalidades estructurales cromosómicas y mutaciones en oncogenes específicos y genes supresores de tumores (APC, PIK3CA, SMAD4, KRAS, TP53, BRAF)		PIK3CA, SMAD4, BRAF sin alterar Sobre-expresión de KRAS, TP53	[29-31]

Tabla 2. Genética del cancer colorrectal e impacto potencial de la diabetes mellitus en los genes relacionados con dicho cáncer.

Los tumores de colon derecho e izquierdo muestran patrones epidemiológicos, sensibilidad a quimioterapia basada en fluoropirimidinas y pronósticos diferentes, probablemente en relación con características moleculares distintas y con la inestabilidad cromosómica que se asocia los tumores de colon izquierdo. Estas características de CCR serán desarrolladas más adelante al hablar de los subtipos moleculares [32].

1.2.2. Carcinogénesis del CCR:

En la década de los 90 fueros descritos dos modelos de carcinogénesis de CCR. Por un lado, el modelo de Vogelstein [23], primer modelo que demostraba que la carcinogénesis humana seguía un patrón multifactorial y multisecuencial, también denominado supresor o de inestabilidad cromosómica. Este modelo está caracterizado por mutación en oncogenes y genes supresores como APC, RAS, P53 y otros, lo que da lugar al fenotipo más común de CCR. Por el otro lado, el modelo publicado de forma paralela por Manuel Perucho [33], en el que existen alteraciones en los genes que codifican las proteínas encargadas de reparar los errores del emparejamiento de bases (Mismatch repair system o MMR) que pueden ocurrir durante la replicación del DNA; los genes de este sistema más importantes son MLH1, MSH2, MSH3, MSH6 , PMS1 y PMS2. Este modelo fue denominado fenotipo mutador, ya que el hecho de no reparar un error de 1-2 nucleótidos mal emparejados da lugar a una sucesión de millones de errores acumulados en el marco de lectura del DNA. Si la alteración de estos genes de reparación es heredada, estamos frente a un Síndrome de Lynch o CCR hereditario no polipósico. Pero también puede ocurrir no en la línea germinal, sino en células somáticas adultas, generalmente por hipermetilación, y por tanto inactivación del promotor de MLH-1 (en ese caso está relacionada con tumores esporádicos con este fenotipo también llamado fenotipo metilador). Los tumores ocasionados por este segundo modelo, bien sea por alteración germinal o somática, tienen características fenotípicas diferentes al fenotipo primero, es decir, están más frecuentemente localizados en colon derecho, tienen mejor pronóstico aunque presentan mala respuesta a 5-FU y derivados.

Una manera indirecta de evaluar los tumores es mediante el estudio de los microsatélites, estudiando como varían las bandas de DNA en un gel (microsatélites: secuencias repetitivas del DNA no codificante, que varían si existen errores del marco de lectura). Según la inestabilidad de estos microsatélites (MSI), los tumores se clasifican en fenotipo metilador e hipermutado, que presentan inestabilidad de microsatélites, y en no hipermutados, con estabilidad en los microsatélites pero con inestabilidad cromosómica.

En los años posteriores se realiza un esfuerzo colaborativo de múltiples instituciones (The Cancer Genome Atlas Network-TCGA) para estudiar y caracterizar diferentes tipos de neoplasias

desde el punto de vista molecular con diferentes aproximaciones: expresión génica, variación de número de copias y alteraciones cromosómicas, cambios epigenéticos (miRNA, metilación, proteómica, transcriptómica, etc). Es el amplio concepto de la biología de sistemas [24].

Como resultado de esta colaboración, se han descrito al menos 5 subtipos moleculares de CCR, con implicaciones pronósticas y terapéuticas. La tabla 3 muestra el resumen de estas clasificaciones moleculares que intentan definir biomarcadores moleculares encaminados a la personalización individualizada de los tratamientos [34].

La figura 1 muestra el paralelismo del modelo genético clásico con los subtipos moleculares, y la figura 2 el acuerdo propuesto entre los distintos subtipos moleculares de algunas de las diferentes clasificaciones propuestas para el CCR.

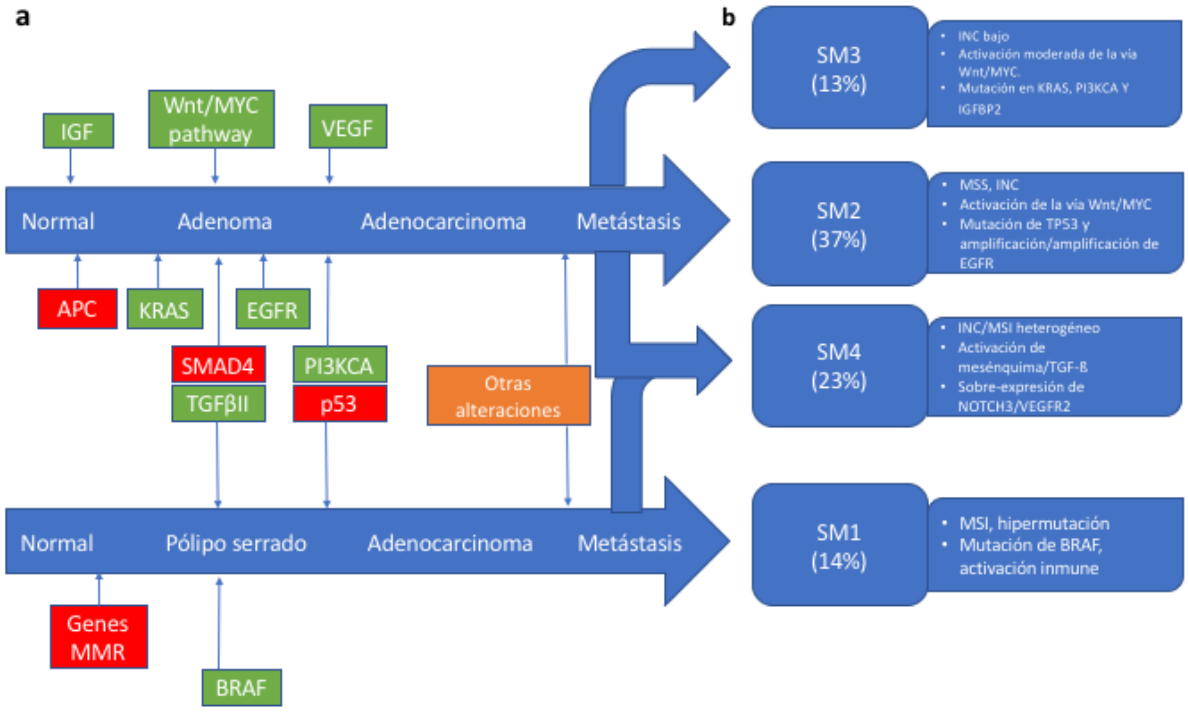

Fig. 1. Correlación entre el modelo genético clásico (a) y los subtipos moleculares (b) en el proceso de carcinogénesis del cáncer colorrectal [34]. INC: Inestabilidad cromosómica; SM: subtipo molecular; PI3KCA: fosfatidil inositol 3 quinasa subunidad catalítica alfa; MSI: inestabilidad de microsatélites; TGF-ß- Factor de crecimiento transformante ß; MMR- Mistmach repair system- Sistema reparador de desequilíbrio; VEGF- Vascular endothelial growth factor-Factor de crecimiento endotelial; EGFR- Epidermal growth factor receptor- Receptor del factor de crecimiento epidérmico.

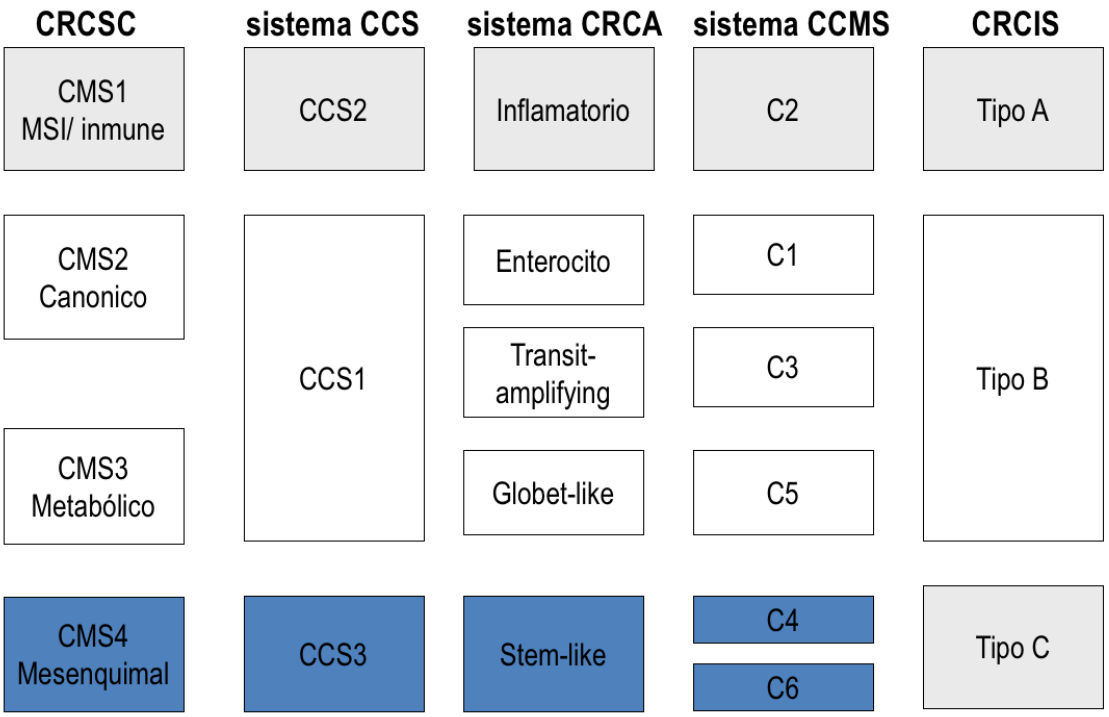

Fig. 2. Acuerdo propuesto entre los distintos subtipos moleculares de algunas de las diferentes clasificaciones propuestas para el CCR [34]. CRCSC: Colorectal Cancer Subtyping Consorcium; CCS: Colon Cancer Subtye system; CRCA: Colorectal Cancer Assigner system; CCMS: Colon Cancer Molecular Subtype system; CRCIS: Colorectal Cancer Intrinsic Subtypes

Autores/año	Nombre	Tipo de validación / análisis	Clasificación	Comentarios
De Sousa E. Melo et al. (2013) [35]	Colon cancer subtipes system (CCS)	Investigación del perfil transcriptómico de 90 muestras de CCR que posteriormente se validaron en 1100 pacientes	CCS1 (49%): mutaciones en KRAS y TP53. CCS2 (24%): fuerte fenotipo metilador. Abundante infiltrado inflamatorio y colon derecho. CCS3 (27%): pueden mostrar MSI y CIN, aunque se caracterizan por la sobre-expresión de genes relacionados con EMT, remodelación del la matriz y migración celular.	Los subtipos CCS1 y 2 muestran similitudes con subtipos ya presentados en otras clasificaciones. El subtipo CCS3 representa un subtipo novel con un agresivo comportamiento y pobre supervivencia
Sadanandam et al. (2013) [36]	Colorectal cancer assigner (CRCA) system	Análisis del perfil de genes de expresión en 1290 CRC de repositorios pertenecientes a diferentes estudios.	Stem-like: Sobre-expresión de los genes de la vía Wnt con características de stem cell mioepiteliales y mesenquimales. Goblet-like: Elevada expresión de miRNA MUC2 y TFF3 Transit-amplifying: grupo heterogéneo con expresión de genes relacionados con stem cell y con la vía Wnt. Se subclasifica en 2 grupos según respuesta a cetuximab. Inflamatorio: Elevada expresión de genes relacionados con citoquinas e interferón. Enterocito: Elevada expresión de genes específicos de enterocitos.	Importantes implicaciones terapéuticas y de valor pronóstico, ya que cada grupo presenta una SLE diferente y distinta respuesta a los fármacos utilizados. Además representa los distintos grados de diferenciación del epitelio intestinal
Marisa et al. (2013) [37]	Colon cancer molecular subtype (CCMS) system	Análisis de una cohorte multicéntrica de 750 tumores frescos congelados	C1 (21%): CIN, mutaciones en KRAS y TP53, supresión de las vías asociadas al sistema inmune y a EMT. C2 (19%): MSI, CIMP, mutaciones en BRAF, supresión de la vía Wnt y activación de las vías de proliferación y de activación del sistema inmune. C3 (13%): MSS, mutaciones en KRAS y delecciones en las vías asociadas con activación del sistema inmune y EMT. C4 (10%): Muestra CIN y CIMP, mutaciones frecuentes en KRAS, BRAF y TP53. Sobre-expresión de las vías asociadas a EMT. C5 (27%): marcada CIN y frecuente mutación en KRAS y TP53, sobreexpresión de los genes de Wnt. C6 (10%): CIN, mutaciones en KRAS y TP53 y frecuente expresión de genes relacionados con EMT y con vías de activación de neoplasias dentadas.	Los subtipos C2,C3, C4 y C6 son clasificados como clusters individualizados, aunque se ven solapamientos entre C1 y C5. Los subtipos C4 y C6 muestran claramente peor pronóstico que el resto de subtipos.
Roepman et al. (2013) [38] Salazar et al. (2011) [30]	Colorectal cancer intrinsic subtypes	Serie previamente analizada de 188 pacientes con CCR, validada en 543 pacientes estadios II y III, realizando un análisis integral del genoma	Tipo A (MMR- deficient epitelial subtype, 22-35%): Frecuente MSI y elevada tasa de mutación, incluido BRAF. Buen pronóstico. Tipo B (epitelial proliferative subtype, 52-62%): fenotipo epitelial con elevado índice de proliferación, todos con MSS y ausencia de mutaciones en BRAF. Pronóstico intermedio. Se benefician de QT adyuvante. Tipo C (13-17%):expresa EMT. Peor pronóstico y no se benefician de QT adyuvante.	Aporta incongruencias comparándolo con las clasificaciones previas.
[34]	Colorectal cancer subtyping consortium (CRCSC)	Análisis de 4000 muestras de CCR	CMS1: Tumores hipermutados con baja prevalencia de alteraciones en el número de copias somáticas que muestran MSI/CIMP, infiltración inmune y frecuentes mutaciones en BRAF. CMS2: Tumores MSS, con CIN e intensa activación de las vías Wnt y MYC, amplificación de EGFR y sobreexpresión de TP53 mutado. CMS3: Baja CIN, elevada prevalencia de CIMP, moderada activación de WNT/MYC, mutaciones en KRAS y PI3K y sobre-expresión de IGBP2. CMS4: CIN, heterogéneos, que expresan características mesenquimales, con activación de TGF-ß y evidencia de vías de angiogénesis activas. Suelen aparecer en estadios avanzados.	CMS1 suele encontrarse en lesiones del lado derecho, CMS2 en izquierdo y recto.CMS4 asocia peor pronóstico con tendencia a la recurrencia y a cortas supervivencias. CMS1 muestra buen pronóstico. Un 13% de las muestras mostraron características mixtas compatibles a dos o más subtipos.

Tabla 3. CCR: Cáncer colorrectal. EMT: Epithelial to mesenchymal transition; SLE: supervivencia libre de enfermedad; MSI: Inestabilidad de microsatélites; MSS: Estabilidad de microsatélites. CIN: Inestabilidad cromosómica ; CIMP: Fenotipo metilador CpG island. MMR: Sistema reparador de desequilibrio. QT: Quimioterapia.

En 2012 se publicó la caracterización que TCGA hace de neoplasias de colon y recto tras estudiar un total de 276 muestras de CCR. Un 16% de los tumores estudiados se caracterizan por ser hipermutados. Tres cuartas partes de ellos tienen silenciado MLH1 por hipermetilación del promotor. Este hecho está asociado a la presencia de mutación V600E de BRAF, que da lugar a una activación constitutiva de la actividad serin-treonin-kinasa de BRAF y por tanto activa la vía de la mitogen activated protein kinase-MAPKinasa (Ras-Raf-MAP-Erks) que finalmente transmite señal mitogénica al núcleo. Del 16% de los casos hipermutados, un tercio de ellos están relacionados con la mutación en línea germinal de los genes del sistema MMR-System que son los casos de Síndrome de Lynch o CCR hereditario no polipósico.

Estas neoplasias hipermutadas aparecen con más frecuencia en colon derecho, tienen en general un mejor pronóstico, salvo que recaigan. Se ha visto que este fenotipo hipermutado, una vez recae, tiene peor pronóstico, ya que son neoplasias más desdiferenciadas, mucinosas, con mutaciones en BRAF y mala respuesta a tratamientos con fluoropirimidinas.

El 84% de las muestras estudiadas no son hipermutadas. En estos casos no se observan diferencias moleculares entre las que aparecen en colon y las que aparecen en recto. La diferencia molecular está entre colon derecho y colon izquierdo incluyendo recto, lo cual es congruente con el origen embriológico del mismo: intestino medio o posterior [24].

Sea cual sea el mecanismo, la acumulación de múltiples alteraciones genéticas conlleva un crecimiento selectivo de las células epiteliales que se transforman moduladas por cambios epigenéticos. El desencadenante de esta acumulación de alteraciones es aún desconocido. Potenciales responsables podrían ser factores dietéticos, del estilo de vida, de la microbiota y la respuesta inflamatoria a esa microbiota [39-44]. Una vía molecular bien conocida para promover la expresión de los genes de proliferación es la Wnt/ß-catenina. La pérdida de función de las mutaciones de APC, como se observa en la poliposis adenomatosa familiar y en el CCR esporádico, o la silenciación epigenética de APC, conlleva la acumulación nuclear aberrante de ß-catenina y la proliferación celular descontrolada. La proteína APC normal es un supresor de tumores que forma un complejo con la quinasa glucógeno-sintasa 3-ß (GSK-3ß) que permite que GSK-3-ß fosforile a ß-catenina. Este proceso conduce a su ubiquitinación y degradación proteosómica, lo que disminuye los eventos transcripcionales dependientes de ß-catenina [45].

1.2.3. Tratamiento del CCR:

El tratamiento del CCR está basado en la cirugía para los estadios precoces y cirugía con quimioterapia adyuvante para los estadios avanzados (estadio III y estadio II de alto riesgo, definido éste como casos de debut como obstrucción o perforación, tumores con pobre diferenciación, invasión vascular, linfática o perineural, CEA preoperatorio elevado y/o T4). El 20-30% de los casos son diagnosticados en estadios avanzados. Y las recaídas ocurren en el 40-50% de los estadios iniciales. El tratamiento estándar del cáncer de recto medio e inferior > T3 o con afectación ganglionar incluye quimioradioterapia neoadyuvante con el fin de mantener el esfínter anatómico y de conseguir el mejor control local [46]. Los esquemas de quimioterapia adyuvante incluyen fluoropirimidinas y oxaliplatino. El tratamiento del CCR metastásico está basado en agentes quimioterápicos como irinotecán u oxaliplatino combinado con fluoropirimidinas y leucovorin (regímenes FOLFIRI o FOLFOX). Estos esquemas han mostrado excelentes resultados como terapia de primera línea [47]. La aparición de las terapias dirigidas en la última década han mejorado aún más la supervivencia de este grupo de pacientes. En la actualidad se testan las mutaciones de KRAS, NRAS y BRAF para predecir el pronóstico y determinar un posible beneficio clínico tras la administración de dianas terapéuticas como cetuximab y panitumumab. Los meta-análisis sugieren que la mutación en el exon 2 de KRAS es el biomarcador de no respuesta a antiEGFR más robusto. Añadir un agente antiangiogénico (anti-Vascular Endothelial Growth Factor-anti VEGF) (bevacizumab or aflibercept) a la quimioterapia de primera o de segunda línea en el CCR metastásico prolonga la supervivencia libre

de progresión (SLP) y la supervivencia global (SG) [48,49].

Clásicamente, el CCR ha sido clasificado según sus características clínico-patológicas. Sin embargo, en situaciones de similares características histológicas y a igual estadio tumoral, el pronóstico y la respuesta a diferentes drogas muestran heterogeneidad. Estas diferencias pueden ser parcialmente explicadas por eventos moleculares de iniciación de CCR como la descrita MSI y las mutaciones en RAS y BRAF. El estadio según TNM y la presencia de MSS nos informan de la necesidad de administrar terapia adyuvante. El estado mutacional de K/N-RAS nos ayuda en la decisión de administrar drogas anti-EGFR en el CCR metastásico. El estado de BRAF añade información pronóstica, aunque su valor como predictor de la respuesta a los agentes anti- Epidermal growth factor receptor (Receptor del factor de crecimiento epidérmico-EGFR) no está clara. Aún así, estos biomarcadores no reflejan la diversidad y complejidad de este tumor y aún no son útiles para realizar terapias individualizadas.

1.3. Asociación epidemiológica entre la DM y el CCR

Algunos estudios epidemiológicos sugieren que la DMT2 está asociada con un mayor riesgo de padecer cáncer en diferentes localizaciones, incluido CCR [50]. La primera asociación prospectiva fue reportada en 1998 con 850.000 participantes estadounidenses, con una edad media de 52 años y un seguimiento entre 1960 y 1972 [51]. El ratio de densidad de incidencia ajustada de CCR fue de 1,30 (intervalo de confianza del 95% 1,03-1,65) en varones diabéticos, pero no fue significativo en mujeres. En varones, se encontró asociación sólo en no fumadores. Un estudio prospectivo más reciente en EEUU en pacientes discretamente más mayores (edad media de 62 años), en una cohorte de 484.020 individuos entre 1995 y 2004, observó un aumento ajustado del HR para CCR en varones (HR 1,23) y en mujeres (HR 1,36) [52]. La edad más avanzada de la cohorte no influyó en las diferencias debidas al género ya que no se observó aumento del riesgo en mujeres mayores de 60 años en el primer estudio [51]. Por lo tanto, la hipótesis que debe considerarse es que cambios en el estilo de vida entre los 60 y los 90 años de edad podrían explicar el cambio del riesgo en mujeres. Una asociación similar fue observada en Japón (HR 1,4; 95% CI, 1,19-1,64, n=336.000) [53], China (tasas de incidencia estandarizada-RIE) para cáncer de colon y recto 1,47 (1,29-1,67), y 1,25 (1,09-1,43) en varones frente a 1,33 (1,15-1,54) y 1,29 (1,10-1,51) en mujeres (n=327.268 pacientes diabéticos tipo 2 seguidos entre 2007 y 2013) [54]; Australia con un RIE para CCR 1,18 en varones frente a 1,16 en mujeres) con 953.382 participantes del registro nacional de diabetes entre 1997 y 2008 [55] o ciertos países europeos como por ejemplo Suecia [56].

Un reciente meta-análisis de estudios observacionales en DMT2 y actualización de cáncer concluyó que el CCR fue una de las 4 localizaciones de cáncer asociada a DMT2 con una evidencia robusta y sin indicio de sesgos [57]. El riesgo relativo fue de 1,27 (1,21 a 1,34), en el rango de meta-análisis previos. Además, un meta-análisis de estudios de cohortes prospectivos que incluyeron cerca de 1 millón de participantes revelaron que la prediabetes (glucosa alterada en ayunas o tolerancia a la glucosa alterada) estaba asociada con un riesgo elevado de CCR [58]. Sin embargo, las incertidumbres permanecen actualmente sin resolver. En el estudio del registro de diabetes en Australia, el riesgo de cáncer estaba significativamente elevado a lo largo del tiempo de seguimiento, siendo más alto en los 3 primeros meses post-registro, lo que sugiere la presencia de sesgos o de causas reversibles [55]. Resultados similares fueron reportados en Israel [59] y en un estudio prospectivo holandés, en el que se encontró un elevado riesgo de cáncer en aquellos pacientes diagnosticados de DM en los tres meses antes del diagnóstico de cáncer, lo que sugiere la potencial influencia de sesgos protopáticos [60]. En este sentido, en un estudio americano, los encuestados con diabetes estaban un 22% más dispuestos a realizar screening de CCR que aquellos sin diabetes [61]. También se ha descrito un riesgo más elevado de desarrollar DM dentro de los 5 primeros años del diagnóstico de CCR [62]. Además, existen diferencias regionales; en Noruega sólo las mujeres con diabetes mostraron una mayor incidencia de

CCR (población total de 751.922 personas-año, edad media de 49 años; RR en varones 0.66 (0.35-1.24); RR en mujeres 1.55 (1.04-2.31)) [63], mientras que no se encontró asociación en El Tirol con un RIE en mujeres 0.94 (0.62-1.36) frente a 1.11 (0.81, 1.49) en varones (5.709 pacientes con DMT2 comparados con la población tirolesa general). En un gran estudio (n=120.852) prospectivo holandés se demostró que el riesgo de cáncer de colon proximal estaba aumentado en mujeres con DMT2 versus en mujeres sin DMT2 (HR 1.80, 95% IC 1.10-2.94), aunque no se encontró asociación entre la DMT2 y el riesgo global de CCR ni en varones ni en mujeres [64]. Además, los datos epidemiológicos procedentes de países en desarrollo son escasos. Esta es una parte importante de pérdida de información ya que aproximadamente el 55% de los casos de CCR ocurren en los países más desarrollados (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx), mientras que el 80% de los pacientes con DM viven en países con rentas medias-bajas (www.diabetesatlas.org/).

Existe un número de factores de riesgo que podrían ser factores de confusión en estudios epidemiológicos, factores a su vez relacionados entre sí, y que son comunes al CCR, a la DM y a los órganos diana de la diabetes ejemplificados en la nefropatía diabética (NFD) (Tabla 4). Los principales factores de confusión son los siguientes:

– **Obesidad:** La obesidad como factor de riesgo para sufrir un cáncer es conocida, y muchos de los tumores más prevalentes (mama, CCR y próstata) están asociados a este riesgo [65]. La obesidad es también el mayor de los riesgos asociado a la DMT2 y al riesgo de CCR. En 5,24 millones de europeos estudiados, el índice de masa corporal (IMC) estaba asociado con CCR (HR 1.10, 1.07-1.13 por cada 5 Kg/m2 de aumento en el IMC), incluso después del ajuste por DM. Este efecto es mayor en varones. La obesidad promueve resistencia a la insulina, efecto que parece que puede ser el mayor responsable de la epidemia actual de DM. Asumiendo causalidad, 10% o más de los cáncer de colon podrían ser atribuibles al exceso de peso [66]. Sin embargo, los estudios clave que observan la asociación entre DM y CCR están ajustados según el IMC. En este sentido, debe existir una relación entre la obesidad, la resistencia a la insulina y el CCR. En un estudio europeo prospectivo entre individuos con sobrepeso, se observó un riesgo menor de cáncer en pacientes con sobrepeso metabólicamente saludables comparados con los metabólicamente no saludables (OR 0.69, 95% IC 0.49-0.96), definidos según niveles elevados de péptido-C indicando hiperinsulinemia [67].

  La información relativa al pronóstico a largo o corto plazo de pacientes diabéticos diagnosticados de CCR es limitada. Los niveles de hemoglobina glicosilada elevados podrían ser un predictor independiente de agresividad clínica en pacientes con CCR avanzado y podría estar asociado con mayor localización derecha, con una pobre supervivencia a los 5 años [68]. La DM por sí misma no parece ejercer alguna influencia en la supervivencia global cáncer-específica en pacientes con CCR. La supervivencia global más baja en los pacientes diabéticos con CCR en comparación con los no diabéticos parece estar asociada con más probabilidad a enfermedad cardiovascular y a la edad elevada [69].

– **Dieta:** La dieta es otro importante factor de confusión que ha sido revisado en diferentes estudios. Dietas ricas en grasas y carnes rojas y usualmente pobres en harinas de cereales no refinadas y fibra parece que aumentan el riesgo de CCR [70]. Los países desarrollados, con mejor situación económica y mayor urbanización, reportan tasas crecientes de obesidad y de síndrome metabólico. El movimiento ocurrido en las últimas décadas en los países de Europa del este durante su transición hacia economías de mercado más abiertas ha permitido una mayor disponibilidad de alimentos que se ha traducido en un aumento de la obesidad. Igualmente, en países del este de Asia, los cambios en la dieta y el aumento de la obesidad han precedido un aumento de la incidencia de CCR [71]. Diferentes estudios han encontrado asociación entre los distintos hábitos alimenticios y las características

moleculares de los tumores de colon y recto. Por ejemplo, el consumo excesivo de grasas parece estar asociado a CCR p53 negativo [72]. También el consumo excesivo de carnes rojas y de alimentos ricos en glucosa se asocia a CCR con mutaciones en p53. Con relación al estado de KRAS, parece que el consumo de vegetables crucíferos podría estar asociado a una menor incidencia en mutaciones de KRAS [73]. Existen estudios que asocian los subtipos moleculares a efectos de determinadas dietas pero deberíamos decir que sus resultados aún no son concluyentes [70]. El consumo elevado de alcohol y bajo de fruta y vegetales está correlacionado con obesidad, consumo de tabaco y escaso ejercicio. Debido a esta estrecha relación, los estudios que relacionan DM y CRC deberían de estar ajustados no sólo por el IMC, sino también por estos factores [74].

- **Ejercicio físico:** El sedentarismo o en general el descenso de la actividad física sumado a factores dietéticos se asocian a riesgo elevado de obesidad, síndrome metabólico, intolerancia a la glucosa, DM y dislipemia, así como a una mayor incidencia de adenomas de colon y de CCR [75]. La actividad física es un factor determinante del gasto de energía y, por lo tanto, del equilibrio energético y el control del peso. El ejercicio físico reduce el riesgo relacionado con las enfermedades cardiovasculares y la diabetes y presenta ventajas considerables en relación con muchas enfermedades, además de las asociadas con la obesidad. De acuerdo a la literatura publicada existe la evidencia que el incremento de la actividad física reduce el riesgo de padecer cáncer, de manera convincente, en el cáncer de mama (especialmente en mujeres postmenopáusicas) y en el cáncer colorrectal [76,77]. Algunos factores que podrían explicar el efecto protector de la actividad física en el cáncer y la DM son la reducción de la grasa corporal, la disminución de los niveles de glucosa e insulina, el aumento de la respuesta inmune, la reducción de la respuesta inflamatoria, reducción de estrógenos y andrógenos y aumento del tránsito intestinal (reducción de la exposición a carcinógenos).
- **Enfermedad renal crónica:** Otro posible factor es la enfermedad renal crónica como resultado de la NFD. Sin embargo, mientras que la NFD está asociada con un aumento de riesgo de cáncer en distintas localizaciones, esto no ocurre con el CCR.

Factor de riesgo	DMT2	Cáncer colorrectal	Nefropatía diabética
Raza	**Afroamericanos, Americanos nativos**	**Afromericanos**	**Afroamericanos, Americanos nativos**
Obesidad	Sí	Sí	Sí
Inflamación	Sí	Sí	Sí
Microbiota	Sí	Sí	Desconocido
Déficit de vitamin D	Sí	Sí	Sí
Dieta rica en proteínas	Sí	Sí	Sí
Dieta pobre en fibra	Sí	Sí	No aplica
Pobre ingesta de magnesio/hipomagnesemia	Sí	Sí	Sí
Angiotensina II	Sí	Sí	Sí
Edad	Sí	Sí	Confuso

Tabla 4. Factores de riesgo claves para DMT2, cancer colorectal y complicaciones de la DM (nefropatía diabética fundamentalmente)

1.4. Potenciales mecanismos de asociación moleculares entre DM y CCR

La asociación entre DM y CCR puede ser el resultado de factores de riesgo comunes entre estas dos enfermedades. Sin embargo, algunos datos epidemiológicos sugieren que la hiperinsulinemia, la hiperglicemia y el tratamiento de la DM son potenciales factores contribuyentes (Figura 3) [78], [79]. Adicionalmente, el microambiente de la DM, rico en productos finales de la glicolisis (PFG), hiperlipidemia, inflamación local/estrés oxidativo, alteraciones en la matriz extracelular y alteración de la microbiota o isquemia debido a la vasculopatía, podría reclutar mediadores secundarios dañinos. En este sentido, existen evidencias en cultivos celulares (tabla 5) y en modelos animales que apoyan un papel directo de la concentración elevada de glucosa, los PFGs, la insulina y la microbiota en CCR.

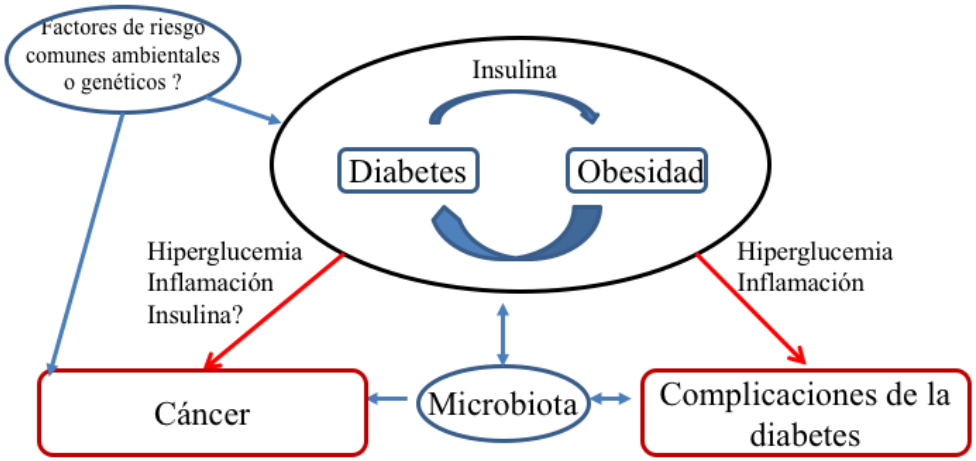

Fig. 3. Posibles factores de riesgo contribuyentes a la diabetes y al cáncer colorrectal

Modelo celular	Intervención	Principales resultados
SW480	HG (11mM) e insulina 100 ng/ml versus glucosa 5.5 mM	HG aumenta proliferación y motilidad
SW480	HG (11mM) e insulina 100 ng/ml versus glucosa 5.5 mM	HG aumenta migración a través de Akt y fosfolipasa C
DLD-1	HG (25mM) versus 5.5 mM de glucosa	HG reduce glutation e incrementa iNOS probablemente a través de la activación de NF- κB
SW480, Sw620, LoVo, HCT116	5-FU con (HG 15mM) versus glucosa 5 mM	HG reduce apoptosis e inhibe la proliferación celular inducida por 5-FU
HCT116	Insulina	Aumenta el tamaño celular
NL-17 y NL-44	Insulina	Aumenta el tamaño celular
Caco-2	PFG: Nε – (carboximetil) lisina (Cas-CML)	Activación de p44/42 (ERK1/2) MAPK
HCT116	PFGs	Incrementa la proliferación celular

Tabla 5. Estudios en cultivos celulares que apoyan un papel directo de la concentración elevada de glucosa, los PFGs, la insulina y la microbiota en CCR. HG: hiperglucemia; 5-FU: 5- fluorouracilo; NF- κB: Factor de transcripción nuclear factor-kappa B; MAPK- Mitogen activated protein kinase- proteínas quinasas activadas por mitógenos; PFGs- Productos finales de la glicolisis; CCR: Cáncer colorrectal

La interacción entre DM y CCR ha sido estudiada en modelos animales con DMT1 y DMT2. Igual que en los humanos, los modelos animales de DMT2 se caracterizan habitualmente por la obesidad. Esto hace difícil separar la contribución específica de este factor en la DM y el cáncer. Sin embargo, desde que se comenzó a relacionar un aumento de riesgo de padecer cáncer con la DM, los modelos animales con DMT2 reflejan con más fidelidad la situación en humanos. Ningún síndrome monogénico asocia diabetes y CCR en humanos. Sin embargo, en trabajos preclínicos, moléculas tales como la resistin-like molecule ß en ratones, aumentan la expresión colónica de las citoquinas T helper tipo-2 y de la IL-17, además de incrementar la susceptibilidad al desarrollo de inflamación, cáncer de colon e intolerancia a la glucosa [88].

1.5. Mecanismos celulares y moleculares compartidos por los órganos diana en la DMT2 y el CCR

La patogénesis del daño a órganos diana en la diabetes y del cáncer de colon es compleja, ya que intervienen numerosas vías de señalización molecular activadas en respuesta a unos factores gatillo clave, tales como la hiperinsulinemia, hiperglucemia e inflamación, entre otros.

1.5.1. Insulina.

La insulina y el factor de crecimiento insulina-like (IGF)-1 tienen propiedades anti-apoptóticas y de inducción del crecimiento en cultivos celulares tumorales y no tumorales. Estas acciones no son específicas para el epitelio del colon ni para células tumorales de colon, pero son interpretadas como parte del supuesto efecto potenciador tumoral de la insulina. Se ha sugerido que la insulina es capaz por sí sola de inducir tumorigénesis directamente activando los receptores de insulina/IGF-1 [89], o indirectamente bajo la influencia de otros moduladores como hormonas sexuales y adipoquinas [90]. La insulina y el IGF-1 promueven la proliferación del epitelio normal del colon in vivo e in vitro [91], además de aumentar potenciales mutaciones espontáneas [92]. La insulina, análogos de la insulina y el IGF-1 inducen proliferación y crecimiento celular y disminuyen la apoptosis en líneas celulares de cáncer de colon [85,92-95]. En este sentido, la insulina activa MAPKs y la vía de señalización RAS-RAF, que promueve proliferación celular, y la vía fosfatidil-inositol-3-quinasa (PI3K)/Akt , que inhibe apoptosis y promueve la síntesis de proteínas [95]. La insulina, además, aumenta la expresión del oncogen MYC, que codifica un factor de transcripción que promueve el fenotipo tumoral y aumenta la proliferación de células de cáncer de colon. Las vías intracelulares mTOR y proteina quinasa-1 activada por p21 (p21-activated protein kinase-1-PAK-1/ Wnt/ß-catenin) están implicadas en la expresión de proto-oncogenes activados por la insulina en cultivos de células intestinales [96,97] e in vivo. Estas vías moleculares también están implicadas en las complicaciones de la diabetes en órganos diana, incluida la NFD [98].

El papel de la hiperinsulinemia ha sido estudiado en crecimiento de cáncer de mama, aunque no hay datos experimentales en animales con cáncer de colon. La vía de señalización de la insulina, de IGF-1 y 2 o la hormona del crecimiento convergen en mTOR. El bloqueo farmacológico de mTOR produce la abolición de la progresión tumoral en células de cáncer de mama en ratones [99]. Sin embargo, no hay evidencia experimental como promotor del cáncer de colon in vivo.

1.5.2. Hiperglucemia.

La hiperglucemia ha estado implicada tanto en crecimiento de cáncer de colon como en la NFD y algunos de los mecanismos moleculares son compartidos por las dos enfermedades. Elevados niveles de glucosa aumentan la proliferación y la migración en cultivos celulares de cáncer de colon [100]. Las vías del polyol y de la hexosamina, que incrementan la oxidación de la glucosa, se encuentran también sobre-expresadas en células epiteliales diana [98] en diabetes y en cáncer de colon [101]. Los PFGs inducen stress oxidativo e inflamación, lo cual puede dañar componentes celulares (ADN, proteínas y lípidos), contribuyendo directa o indirectamente a la transformación celular maligna [102,103]. Los PFGs estimulan la proliferación de células cultivadas de cáncer de colon vía activación y aumento de expresión de la proteína de unión al

elemento de respuesta a carbohidratos (carbohydrate response element-binding protein-ChREBP) [87], un factor de transcripción clave también implicado en la NFD. El stress oxidativo también juega un papel relevante en el desarrollo de las complicaciones secundarias a la diabetes al activar diferentes vías, incluyendo la protein-quinasa C y el factor de transcripción nuclear factor-kappa B (NF-κB) y la AP-1 [104,105].

El efecto Warburg se refiere al alto consumo de glucosa y metabolismo de la glucosa a través de la glicolisis anaeróbica en vez la fosforilación aeróbica en células tumorales, a pesar de la presencia de oxígeno [106,107] M. La glicolisis es menos efectiva pero produce ATP de manera más rápida, lo que le confiere a la célula tumoral una ventaja para crecer con más rapidez. La sobreexpresión de los transportadores de glucosa insulin-dependientes como glucotransporter-1 (Glut-1) favorece el consumo de glucosa por las células tumorales [108]. La sobre-expresión de Glut habitualmente se traduce en tasas de proliferación más elevadas. Sin embargo, los elevados niveles de glucosa aumentan la expresión de Glut-1 en células de cáncer colorrectal CX-2 con baja proliferación y acelera la síntesis de proteínas en vez de aumentar la capacidad proliferativa [109]. En este sentido, el entorno diabético y TGF-ß1 up-regulan Glut-1 de células renales y se cree que contribuyen a la patogénesis del riñón diabético [110]. Los altos niveles de glucosa también incrementan la resistencia a la apoptosis inducida por 5-fluorouracilo.

Pocos trabajos han estudiado el impacto de la hiperglucemia per se en cáncer de colon en modelos animales. La hiperglucemia inducida por estreptozotocina aumenta el tamaño y el número de focos metastásicos hepáticos en ratones con cáncer de colon, mientras que el control de la glucemia con insulina o con glicacida es protector [111]. Estos estudios sugieren que, en ausencia de insulina, la hiperglucemia per se puede favorecer el crecimiento de tumores colorrectales y que esa hiperglucemia puede ser un potencial estímulo más poderoso para la tumorigénesis que la insulina en animales experimentales.

En células renales, la hiperglucemia y los PFGs promueven la muerte celular y activan respuestas fibrogénicas e inflamatorias, lo que contribuye en el proceso de desarrollo del riñón diabético [112-114]. De forma interesante, la respuesta inflamatoria y fibrogénica en células renales inducida por cifras de glucosa elevadas puede ser prevenida por la activación del receptor de la vitamina D [113]. El déficit de vitamina D es común en la DM [115] e incluso podría estar asociado con un elevado riesgo de desarrollar un cáncer, específicamente CCR, la localización más frecuente de cáncer asociada a una insuficiencia de vitamina D [116,117]. La vía molecular de este mecanismo protector de la vitamina D frente al cáncer es parcialmente conocida. La activación del receptor de la vitamina D antagoniza la señal de Wnt/ß-catenina, fundamentalmente en células de carcinoma de colon en humanos. En cáncer de colon, la activación de ß-catenina es la consecuencia directa de la pérdida de función asociada a las mutaciones del gen APC (figure 3). Wnt/ ß-catenina también es activada en las células renales del riñón diabético [118], activación que protege las células mesangiales glomerulares de la apoptosis mediada por altos niveles de glucemia pero que provoca disfunción de los podocitos y proteinuria. En la enfermedad renal no debida a DM, la acción nefroprotectora de los activadores del receptor de la vitamina D (ARVD) está relacionado con la inhibición de Wnt/ ß-catenina. Además, el índice de proliferación, la expresión de ß-catenina y la fosforilación alterada fueron más elevados en el epitelio normal de colon que rodea tejido tumoral en diabéticos que en pacientes no diabéticos.

El bloqueo genético o farmacológico de EGFR ralentiza experimentalmente la progresión de la enfermedad renal [119]. Niveles de glucosa altos, angiotensina II y los PFGs promueve la transactivación de EGFR en células renales [120] y la inhibición de EGFR con erlotinib atenúa el desarrollo del riñón diabético en DNT1 experimental, el cual es mediado por lo menos en parte por la inhibición de mTOR. La señalización de EGFR contribuye a la tumorigénesis y a la progresión tumoral del CCR y, de hecho, el cetuximab es uno de los tratamientos de CCR.

1.5.3. La inflamación y la microbiota.

la inflamación es también un factor crítico en la inducción del daño a los órganos diana en la diabetes y en la iniciación y progresión del cáncer de colon [6,97]. En algunos modelos

animales de DMT2, la inflamación contribuye a la carcinogénesis y el crecimiento tumoral que son detenidos al administrar anticuerpos monoclonales TNF-neutralizadores en ratones ob/ob [121].

Existen muchas vías de señalización implicadas en la respuesta inflamatoria, incluidas NF-κB, janus quinasa/transductor de señal y activador de la transcripción (JAK/STAT) y el factor inducible por hipoxia-1α [122-126]. La sobreexpresión de la esfingosina-1-fosfatasa (S1P) mediada por la quinasa esfingosina-1 conduce a una amplificación persistente de receptor NF-κB/IL-6/STAT3/S1P que induce el crecimiento y la proliferación de CCR [127]. La vía no canónica NF-κB también parece implicada en las complicaciones de la DM y en el cáncer [128,129]. Además, la quinasa contra corriente de esta vía, NIK, contribuye al mecanismo central de fracaso de las células beta en la obesidad inducida por la dieta [130], promueve daño renal [131] y la activación de NIK, que podría ser la base de la sensibilidad de los ratones N1rp12-/- a la inflamación intestinal y a la tumorigénesis. Estas vías intracelulares coordinan la producción de citoquinas inflamatorias, quemoquinas y prostaglandinas, lo que conlleva la infiltración de macrófagos y linfocitos T reguladores, amplificándose la respuesta inflamatoria y promoviéndose la angiogénesis, el crecimiento tumoral y la invasividad de la células malignas [132,133], así como la progresión del daños en los órganos diana de la diabetes, como es el riñón [126].

La interacción entre las células epiteliales de colon y la microbiota puede conferir susceptibilidad para cáncer de colon y obesidad. El inflamasoma regula la microbiota y la respuesta inflamatoria de las células epiteliales a la microbiota. La deficiencia de algunos componentes del inflamasoma está asociada con una microbiota anormal, respuesta inflamatoria exacerbada [134] y tumorigénesis en el colon [135] dependiente de la señal de activación de la IL-6 epitelial inducida por la microbiota [39]. La respuesta inflamatoria dependiente de la microbiota puede contribuir a la agregación familiar no mendeliana de cáncer de colon, ya que en modelos preclínicos el riesgo de cáncer es transmisible entre individuos que conviven. La microbiota intestinal también impacta en el metabolismo del huésped facilitando la obesidad, la resistencia a la insulina y la DMT2 [136]. Por lo tanto, los cambios en la microbiota intestinal asociados a las deficiencias en el inflamasoma están relacionados con la resistencia a la insulina y la obesidad [137]. La DMT2 y el CCR comparten algunas características en su microbiota, tales como menos bacterias productoras de butirato [138]. El butirato es un producto final de la fibra de la dieta que presenta propiedades antitumorales y que se asocia con una menor incidencia de CCR [40].

1.5.4. Cambios epigenéticos.

El CCR y la DM también comparten cambios epigenéticos. Ambas enfermedades se asocian con un resultado positivo en el análisis de la metilación-ADN de septina 9 (SEPT9) (Epi-proColon). Septina 9 está diferencialmente metilado en islotes celulares de humanos con DM y parece que altera la secreción de insulina y glucagón [139].

Los miRNA patogénicos pueden aparecer compartidos por el CCR y la NFD [140-142]. En la NFD en ratones, la expresión de miR-21 renal se mostró aumentada y el descenso de miR-21 aminoró el daño renal [143]. Sólo algunas publicaciones apoyan el potencial patogénico de miR-21, pero otras son contradictorias [144]. Algunos estudios funcionales apoyan el papel del miR-21 en la proliferación e invasión del CCR [145] y dianas terapéuticas contra miR-21 mejoran las sensibilidad de las células de cáncer de colon humano a la quimio-radioterapia y reducen la angiogénesis [146]. La sinergia de la metformina con el 5-fluorouracilo y el oxaliplatino para inducir muerte o quimioresistencia en células de cáncer de colon está también asociada con una reducción en miR-21 [147].

1.6. Influencia del tratamiento antidiabético en el paciente con cáncer.

La ADA Standards of Medical Care in Diabetes 2014 considera al paciente con cáncer una situación clínica inmersa en comorbilidades, por lo que el manejo de una diabetes de base puede ser complicado e insiste en recomenda-

ciones de detección precoz de cáncer apropiadas según sexo y edad para reducir los factores de riesgo modificables, fundamentalmente obesidad, hábito tabáquico y actividad física [9].

Por otro lado, la ADA en 2016 no realiza recomendaciones específicas de antidiabéticos para pacientes con cáncer o CCR [148-150]. Sin embargo, el antidiabético más recomendado de inicio, la metformina, se ha visto asociado con un descenso de la incidencia o de mejor pronóstico en pacientes con cáncer [151]. Por lo tanto, incluso si se demostrara este efecto en estudios clínicos prospectivos, probablemente no se modificaría su uso clínico para pacientes con DMT2 vigente en la actualidad, tengan o no un tumor asociado. En este sentido, aunque los estudios observacionales sugieren que la metformina podría modificar los riesgos y la supervivencia asociada al cáncer [152], no existen estudios prospectivos específicamente diseñados para medir este efecto. Un reciente meta-análisis con una población aproximada de 7,6 millones de pacientes con diabetes para estudio observacional y 137.540 pacientes de ensayos clínicos randomizados sugiere que el uso de metformina o de tiazolidinedionas podría estar asociado a un descenso del riesgo de cáncer, mientras que la insulina, sulfonilureas e inhibidores de la α-1 glucosidasa podrían estar asociados a un aumento del riesgo de cáncer [153].

La administración de insulina de forma crónica en pacientes con DMT2 parece asociada a un aumento del riesgo de CCR, aunque los resultados publicados muestran controversia [154,155]. Niveles circulantes elevados de insulina también están relacionados con riesgo de adenoma y de apoptosis reducida en la mucosa rectal sana [156]. En un meta-análisis que combina 12 estudios epidemiológicos (7 estudios caso-control y 5 cohortes) en América (5 estudios), Europa (3 estudios), y Asia (4 estudios, la insulina estaba asociada a un significativo aumento del riesgo de CCR en pacientes con DMT2 [157]. Sin embargo, en este tipo de diabetes, la insulina suele ser prescrita de forma tardía, por lo que son pacientes que acumulan ya un riesgo asociado a la edad o a la presencia de enfermedad renal crónica. Por otro lado, el estudio de casos-controles Barcelona con 275.164 pacientes con DMT2 no encontró un riesgo elevado de padecer cáncer con insulina ni con ningún antidiabético [158]. Incluso hay más controversia, ya que un meta-análisis con 19 publicaciones y 1.332.120 pacientes con DMT2 y 41.947 casos de cáncer, la insulina glargina se mostró asociada con un menos riesgo de CCR [159].

1.7. Información adicional desde una aproximación de biología de sistemas.

La aplicación de la aproximación de biología de sistemas aporta información no sesgada de potenciales biomarcadores y de vías fisiopatológicas de significado terapéutico. Los datos disponibles son más útiles si se encuentran reunidos en una misma fuente de información que sea accesible [160].

Los estudios de asociación de genoma integral (Genome-wide association studies -GWAS) identificado genes con susceptibilidad para DM y CCR que ofrecen conocimiento sobre potenciales vías patogénicas compartidas, tales como TCF7L2, KCNQ1, HMGA2, RHPN2 y GREM1.

TCF7L2 alberga variantes genéticas comunes con el efecto más sólido de riesgo de DMT2 y que impacta en algunas complicaciones de la DM tales como la NFD [161]. TCF7L2 es un factor de transcripción y un compañero de transcripción de ß-catenina en la vía Wnt que muestra cientos de sitios de interacción de gran afinidad en las células de carcinoma de colon. El TCF unido al ADN reprime la transcripción genómica en ausencia de ß-catenina [162]. Además, TCF7L2 también promueve la expresión de miR-21. Un polimorfismo de nucleótido sencillo (Single Nucleotide Polymorphism-SNP) asociado a CRC, rs 6983267, está ubicado en el sitio de unión del TCF7L2 y el alelo de riesgo presenta una unión más fuerte al TCF7L2, lo que facilita la señal de Wnt [163]. Un SNP común de GREM1, el rs16969681, asociado con mayor susceptibilidad para CCR, facilita la unión de TCF7L2 con el ADN, lo que tiene como consecuencia una expresión génica más intensa y le confiere a este grupo un riesgo adicional del 20% diferencial con la población general [164]. La duplicación germinal de GREM1 produce el síndrome de poliposis hereditaria y una predisposición mendeliana dominante al

CCR a través de sobre-expresión ectópica de GREM1 en el epitelio germinal [165]. GREM1 fue identificado como uno de los genes más sobre-expresados en cultivos de células mesangiales expuestos a cifras elevadas de glucosa, y GREM1 presenta variantes génicas asociadas a la NPD [166]. Gremlin, la proteína codificada por GREM1, se ha propuesto como un mediador clave en la nefropatía diabética. Esta proteína promueve la motilidad de las células del CCR y la transición de epitelio a mesénquima en las células del túbulo renal, también relacionado con motilidad elevada. Aún así, el papel preciso de TCF7L2 está por concretar en estudios futuros. De momento parece que las mutaciones de TCF7L2 estudiadas en cáncer producen la abolición de su habilidad para funcionar como regulador transcripcional y resulta en un aumento del crecimiento celular del CCR [167].

KCNQ1 es otro de los genes identificados y asociado con DMT2 [168]. Su locus codifica KCNQ1 y el un RNA largo no codificante, que es objetivo de forma alterada de ß-catenina en CCR [169]. En humanos, la expresión de KCNQ1 se asocia a una pobre supervivencia y la mutación de su homólogo en ratones, Kcnq1, a un aumento del riesgo de presentar tumores intestinales [170].

HGMA2 es un gen asociado a riesgo de DMT2 y a NPD en GWAS [171]. Su expresión está aumentada en el CCR [172] y promueve un comportamiento agresivo en estudios experimentales [173].

Las herramientas bioinformáticas intentan integrar las crecientes bases de datos de la biología de sistemas. Una de estas herramientas, la red de vías de señalización droga-específica (Drug-specific Signaling Pathway Network-DSPathNet) ha sido usada para identificar de forma tentativa 7 genes (CDKN1A, ESR1, MAX, MYC, PPARGC1A, SP1 y STK11) y la nueva vía centrada en MYC que podrían jugar un papel en el efecto de la metformina como agente antidiabético y como droga anti-cáncer. Curiosamente, PPARGC1A protege frente al daño renal y su expresión se ve disminuida por la inflamación [174].

1.8. Preguntas sin resolver

La asociación entre DM y CCR está reconocida por consenso científico [78]. Sin embargo, se requieren estudios más detallados para lograr la evidencia.

Un vista general de la incidencia/prevalencia de la DMT2 y el CCR por países sugiere que el ambiente, el nivel de desarrollo entre otros factores pueden interactuar con el ambiente diabético para aumentar el riesgo de CCR. La identificación de esos factores y si la DM se asocia a un incremento del riesgo de padecer CCR en diferentes culturas y países puede aportarnos conocimiento acerca de los mecanismos que subyacen en la posible relación existente entre estas dos enfermedades.

En caso de existir una asociación causal, ésta debería de estar reforzada por una caracterización de las vías moleculares vinculadas en el debut de una DM. Esta información puede llevar al desarrollo de medidas preventivas o terapéuticas. Los estudios deben de analizar la relación entre el CCR asociado a la DM y al desarrollo de otras complicaciones relacionadas con la diabetes, como por ejemplo, si existe un perfil de paciente proclive a desarrollar cualquier complicación relacionada con la DM. En ese caso, los esfuerzos deben de estar encaminados a la identificación precoz de esos pacientes. La identificación precoz de subpoblaciones de pacientes diabéticos en riesgo elevado de desarrollar cáncer o complicaciones clásicas podría permitir el diseño de ensayos clínicos que evalúen la eficacia de drogas que actúen contra dianas moleculares compartidas para la prevención y/o la terapia. La investigación es también necesaria para definir el manejo más óptimo del paciente con DMT2 y CCR.

La aproximación a la biología de sistemas puede ayudar a definir vías moleculares que conducen a cáncer asociado a diabetes o al daño de un órgano diana en diabetes, y permitiría diseñar estudios experimentales que nos lleven a analizar el potencial terapéutico en esas vías para prevenir o tratar ambas enfermedades. Estas propuestas deberían de llevarse a cabo en ensayos clínicos en poblaciones de alto riesgo o en estadios precoces de la enfermedad.

1.9. Epílogo

La epidemiología sugiere una asociación entre la DM y un riesgo elevado de distintos tipos de cáncer. Los resultados de algunos estudios observacionales sugieren que la DM podría aumentar de forma significativa el riesgo de cáncer de colon (CC) [52,54,55,59,64,175]. Sin embargo, los resultados de muchos de estos estudios están argumentados en base a grupos heterogéneos, con datos poco fiables de status de diabetes en muchos casos y utilizando fuentes con poco rigor como registros nacionales (Surveillance, Epidemiology and End Results-SEER), mostrando resultados con conclusiones poco consistentes y confusas [55,176,177]. La falta de bases de datos de pacientes que reúnan datos fenotípicos, clínicos y demográficos es siempre un obstáculo en la investigación clínica. Se requieren grandes esfuerzos para obtener con consentimiento datos clínicos y recoger muestras de tejidos de población de riesgo de desarrollar complicaciones de diabetes y sometidos a pruebas de screening de diagnóstico precoz de cáncer. Cruzar los datos de las muestras obtenidas con datos clínicos es esencial para entender la heterogeneidad de los pacientes con diabetes y cáncer.

Por lo tanto, la magnitud de la asociación entre las dos enfermedades no ha podido ser estudiada con rigor, a pesar de ser enfermedades tan accesibles y prevalentes, y dicha asociación podría haber estado potencialmente influenciada por sesgos.

Resulta además incierto si el supuesto vínculo entre la DM y el CC está directamente relacionado con la hiperglucemia; si la DM es un factor biológico latente que puede modificar los factores de riesgo de cáncer, como puede ser la resistencia a la insulina; o si la asociación diabetes-cáncer es indirecta y refleja la influencia de factores de riesgo comunes como la edad, el estilo de vida, sobrepeso, hábitos dietéticos no saludables o la administración de unos u otros tratamientos [78]. Además, tenemos un conocimiento limitado acerca de la influencia del ambiente diabético en el proceso de carcinogénesis, el comportamiento biológico del tumor, la respuesta a los distintos tratamientos y el pronóstico de estos pacientes [4,178]. Los estudios in vivo e in vitro apoyan un papel directo de las concentraciones elevadas de glucosa en el desarrollo del tumor y de sus características [100]. En este sentido, algunos modelos animales han analizado la influencia de la diabetes/resistencia a la insulina en la carcinogénesis (por ejemplo, las moléculas carcinogénicas usadas en ratones db/db con deficiencia del receptor para leptina) [179], o la influencia de la diabetes/hiperglucemia en la progresión de tumores en ratones xenoinjertados [180] y ratones con diabetes inducida por estreptozotozina [181], aunque el cáncer de colon no ha sido previamente estudiado en profundidad.

2. HIPÓTESIS Y TRABAJO EXPERIMENTAL

2.1. Hipótesis de trabajo

Expuesto lo anteriormente, la hipótesis de este trabajo sostiene que, según apunta la bibliografía existente, existe asociación entre la DM y el cáncer, centrado en el CC como tumor frecuente y con literatura científica suficiente, asociación que se traduce en diferencias clínicopatológicas entre los tumores de pacientes sanos y los tumores que están sometidos al ambiente proinflamatorio de la DM.

Por otra parte, y dado que disponemos de medios para realizar estudios en modelos animales de CCR a los que se puede inducir DM y por tanto realizar estudios de cinética tumoral con o sin DM asociada y hacer una comparación histopatológica en ambos grupos de animales, pensamos una segunda hipótesis: existen diferencias en la cinética tumoral en presencia de DM.

2.2. Objetivos del trabajo

La Fundación Jiménez Díaz ofrece asistencia médica a 450.000 habitantes y todos los datos médicos se encuentran almacenados de forma electrónica. Esto representa una oportunidad para evaluar la posible relación temporal existente entre diabetes tipo II y la incidencia de cáncer y evaluar el impacto de la diabetes en la mortalidad de los pacientes con cáncer. Por este motivo, los objetivos marcados son:

- Generación de una base de datos retrospectiva de pacientes con cáncer colorrectal con y sin diabetes
- Analizar los parámetros clínico-patológicos de los pacientes para evaluar diferencias que puedan ser atribuibles a la diabetes.
- Desarrollar un modelo de xenoinjerto con una línea de cáncer de colon humana en animales atímicos con diabetes previamente inducida con estreptozotocina y un grupo control.
- Evaluar las diferencias en términos de cinética de crecimiento tumoral y de características histológicas en el modelo animal.
- Comparar los resultados obtenidos en ambos abordajes con el fin de aumentar el conocimiento sobre la influencia del ambiente diabético en las características del cáncer de colon.

2.3. Material y métodos

2.3.1. Estudio clínico: muestra poblacional

Este estudio recoge de forma retrospectiva pacientes atendidos en la Fundación Jiménez Díaz entre enero de 2009 y diciembre de 2013. Para su selección se utilizó el software denominado Alcor, desarrollado por Sigesa. Este programa informático trabaja con un sistema de codificación que realiza búsquedas según determinados términos, identificando procesos y números de historia clínica asociados a dicho término. La primera búsqueda se realizó utilizando los términos CCR y DM, y la segunda DM, cáncer y CCR. Hay que decir que el sistema de codificación unifica las dos localizaciones, colónica y rectal, bajo el mismo código, siendo necesario investigar en la historia clínica para conocer la ubicación exacta del tumor. Los datos clínicos de los pacientes seleccionados y sus correspondientes biopsias fueron revisados tras consentimiento informado proporcionado por los pacientes para los objetivos de investigación.

Con los datos disponibles se obtuvieron 81 pacientes con el diagnóstico de CCR y que fueron intervenidos, condición indispensable para disponer de muestra biológica, durante ese periodo de tiempo. Los criterios de inclusión para conseguir una muestra homogénea fueron:

- Adenocarcinoma como tipo histológico.
- Localización en colon (los casos de cáncer de recto fueron excluidos) [182]-171].
- Sin tratamiento neoadyuvante.
- Supervivencia superior a 6 meses.
- Sin otras neoplasias concurrentes o tratamientos inmunosupresores.
- Diagnóstico de DM comprobado y documentado mediante registro de dicha enfermedad en los antecedentes, histórico de toma de

medicación antidiabética, y/o cumplimiento de los criterios definidos por la ADA en el momento del proceso de cáncer. Los criterios utilizados por la ADA para determinar si una paciente padece una diabetes o no son: Hemoglobina glicosilada ≥ 6,5%, glucemia en ayunas ≥ 125 mg/dL con valores elevados medidos en 2 o más ocasiones, o glucemia al azar ≥ 200 mg/dL, con valores elevados medidos al azar en 2 o más ocasiones.

De forma paralela, se reclutaron 79 pacientes no diabéticos con el diagnóstico de CC, que fueron intervenidos durante el mismo periodo de tiempo, utilizando los mismos criterios de inclusión excepto la presencia de DM, con el objetivo de conseguir una serie homogénea y correctamente balanceada.

Las variables básicas recogidas en todos los pacientes incluían edad, género, toma de metformina, forma de debut del CC (aguda o subaguda), estado general medido según el Eastern Cooperative Oncology Group (ECOG) Scale Performance Status, índice de masa corporal (IMC), terapia adyuvante, SG en el momento de recogida de los datos (4 años), SLE y causa de la muerte en los casos en que procediera. Otras variables obtenidas a partir de sangre periférica incluían glucemia, triglicéridos, colesterol, cifras de leucocitos y antígeno carcinoembrionario (CEA).

Las características del tumor se analizaron en base a los siguientes criterios:

- La información relativa a la profundidad de invasión del tumor (pT) se obtuvo del informe anatomo-patológico y se definió según la American Joint Committee on Cancer Criteria para estadificación del CCR .
- El grado de diferenciación del tumor se hizo en bajo grado (G1-G2) y alto grado (G3), siguiendo las recomendaciones de la World Health Organization de 2010 .
- La localización tumoral se categorizó según fuera proximal o derecha (ciego, flexura hepática, colon ascendente y transverso) y distal o izquierda (flexura esplénica y colon descendente) [182].
- Los datos de infiltración linfovascular fueron extraídos del informe anatomopatológico, determinado tras tinción con hematoxilina-eosina. La invasión venosa fue considerada como presente si se observaban células tumorales en el canal endotelial con músculo liso. La invasión linfática se definió como presente si las células tumorales se observaban en los vasos linfáticos.
- La estadificación TNM se realizó tras analizar los datos clínicos registrados y la información adicional de los informes anatomopatológicos después de la resección tumoral y teniendo en cuenta las pruebas de imagen disponibles. Se utilizó la estadificación TNM propuesta por la octava edición del TNM de la American Joint Committee on Cancer . Por criterios analíticos, el estadio T fue agrupado en bajo grado (T1-T2) o alto grado (T3-T4); el estadio N fue considerado N0, en ausencia de afectación nodal) o N+, en caso de afectación ganglionar presente; el estadio final, como bajo grado (0, I, o cualquier II) o alto grado (cualquier III y IV).

El estudio fue aprobado por el Comité Ético e Institucional de la Fundación Jiménez Díaz (CEIC-FJD, código de aprobación 08/13; en 1 de Octubre, 2013) según los principios acordados en la Declaración de Helsinki.

2.3.2. Modelo tumoral de xenoinjerto en ratones con diabetes inducida con estreptozotocina

Se adquirieron a los laboratorios Charles River quince ratones macho atímicos UN-Foxn1nu con 8 semanas, y se estabularon en un ambiente libre de patógenos en el animalario de nuestro centro (Animal Model Core Facility of Research Health Institute – Fundación Jiménez Díaz-ES28079000089). Todos los procedimientos y protocolos experimentales con los ratones fueron aprobados por el Órgano Encargado del Bienestar Animal (OEBA) y por la Dirección General de medio ambiente de la Comunidad de Madrid en cumplimiento de los requerimientos establecidos por el gobierno de España y la Comunidad Europea (Real Decreto R. D. 53/2013).

3.2.1. Inducción de la diabetes:

La diabetes fue químicamente inducida con una única inyección intraperitoneal de 200 mg/

Kg de peso de estreptozotocina (STZ, Sigma-Aldrich) en un volumen total de 200 µl en 50 mM de tampón citrato (pH=4,5) en 10 ratones. El grupo control (5 ratones) recibió 200 µl de tampón citrato. Diez días después de la administración de la estreptozotocina, el 60% de los animales inyectados presentaba niveles de glucosa en sangre por encima de los 200 mg/dl. Este fue el grupo considerado diabetes inducida por estreptozotocina (STZ-D). Los niveles de glucosa fueron monitorizados periódicamente en todos los animales durante el experimento. Los animales no recibieron insulina u otros antidiabéticos.

Implantación de los xenoinjertos:

Se utilizaron para ello líneas celulares humanas de cáncer colorrectal HT29, recientemente clasificados como subtipo metabólico, para generar los xenoinjertos 20 días después de la administración de la STZ o de su vehículo de administración (tampón citrato).

Las células se cultivaron en un medio RPMI-1640 (Gibco) con un 10% de suero bovino fetal a 37°C en una atmósfera al 5% de CO2. Este medio fue suplementado con penicilina G (100U/ml) y estreptomicina (0.1 mg/ml).

Los ratones fueron inyectados subcutáneamente con un volumen de 200 µl con 2x106 células [1:1 mezcla de PBS: Matrigel (BD Biosciences)] en ambos flancos del animal.

De forma periódica, se midió el tamaño del tumor generado en cada flanco tres veces a la semana con un calibre Vernier. Las medidas se tomaron en los dos ejes perpendiculares y el volumen se calculó según la fórmula: volumen = (Longitud diámetro largo x Longitud diámetro ancho2)/2.

Evaluación patológica:

Transcurridos 55 días desde la inducción del tumor, los ratones fueron sacrificados. Los tumores fueron extraídos fijados en formalina al 10% durante 24 horas y posteriormente fueron embebidos en parafina. Todas las muestras fueron procesadas siguiendo el mismo procedimiento. Los tejidos fueron cortados en secciones de 4 µm de grosor y teñidos con hematoxilina-eosina para realizar su examen morfológico al microscopio.

Las estructuras vasculares en el tumor fueron examinadas por inmunohistoquímica mediante el marcador CD31. Se obtuvieron secciones consecutivas de 4 µm de grosor de las muestras parafinadas. Se realizó la recuperación de la expresión de antígeno en un PT-Link (Dako) durante 20 minutos a 95ºC en una solución tampón de alto pH (Dako).

La peroxidasa endógena fue bloqueada con la inmersión de las secciones en peróxido de hidrógeno al 0.03% durante 5 minutos. Las muestras fueron después lavadas durante 5 minutos con una solución salina tamponada con Tris y que contenía Tween 20 con un pH de 7,6 y fueron incubadas con un anticuerpo primario para CD31 (dilución 1:25, Clone JC70A, Abcam) durante 20 minutos a temperatura ambiente, seguido de la incubación con el correspondiente anticuerpo secundario conjugado (EnVision, Dako).

Las secciones fueron entonces visualizadas con 3,3'-diaminobenzidina como cromogéno durante 5 minutos y teñidas con hematoxilina. Todas las tinciones se realizaron con una plataforma automática (Dako). Las estructuras vasculares CD31 se contaron en 10 campos usando un aumento de x200 en el área de mayor densidad vascular del tumor.

2.3.3. Análisis estadístico

Los datos se analizaron con el programa spss v.20.0. Las variables continuas se expresaron como media ± desviación estándar, y las categóricas como frecuencias y porcentajes. Las características demográficas/clínicas y las variables del tumor se compararon entre los distintos pacientes con y sin diabetes mediante el test de Fisher o Chi cuadrado según procediera en caso de variables categóricas; y con test t en el caso de variables continuas. Un valor de p inferior a 0.05 se consideró como estadísticamente significativo en todos los análisis realizados.

2.4. Resultados

2.4.1. Datos epidemiológicos

Entre los 1137 pacientes diagnosticados de CCR entre enero de 2009 y diciembre de 2013, 185 (16%) sufrían DM; la incidencia de cualquier tipo de cáncer entre los 13873 pacientes diabéticos de este periodo fue del 14%, tratándose en el 1.3% de estos casos de CCR (Figura 4).

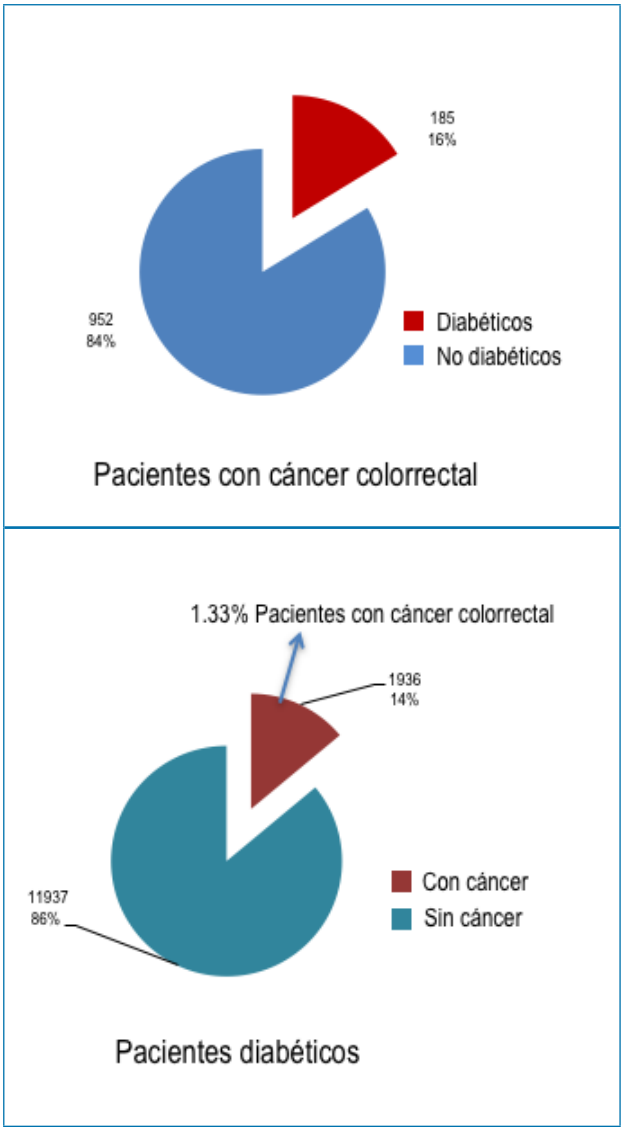

Fig. 4. Muestra poblacional de pacientes con cáncer colorrectal y diabetes utilizada para el estudio epidemiológico

2.4.2. Características del cáncer de colon en pacientes diabéticos versus no diabéticos

Los datos descriptivos, los resultados clínicos y las frecuencias de las variables que constituyen los criterios de inclusión de los pacientes con CC diabéticos y no diabéticos aparecen representados en la tabla 5. Las características demográficas y clínicas en ambos grupos fueron homogéneas respecto al género, edad, debut clínico y niveles de CEA, mientras que los niveles de glucosa, triglicéridos y los neutrófilos circulantes se mostraron significativamente elevados y el colesterol sérico significativamente bajo en diabéticos. El IMC se mostró más elevado con una tendencia no significativa en diabéticos.

El estado ECOG fue 0 el 99 pacientes (61.9%) y ≥1 en el resto de los pacientes (38.1%), mostrándose en el grupo diabético una prevalencia más alta de puntuación ≥1 (ECOG ≥1 en 29.1% en no diabéticos vs 46.9% en diabéticos, p=0.02).

Respecto a las variables relativas al tumor, no se encontraron diferencias significativas entre diabéticos y no diabéticos en la puntuación de T elevada, afectación ganglionar, grado de diferenciación, presencia de invasión linfovascular, localización tumoral, tasa de recurrencia, eventos de muerte o muertes debidas al cáncer.

Del total de pacientes diabéticos, 35 recibían metformina. No se observaron diferencias significativas relativas a las características del tumor entre pacientes diabéticos tratados con metformina o con otros antidiabéticos (Tabla 6).

Variable	Total (N=160)	No diabéticos (N=79)	Diabéticos (N=81)	P
Edad, años	74.8 ± 10.4	72.9 ± 11.3	76.7 ± 9.2	0.02
Glucemias, mg/dl,	112.4 ± 38.5	93.2 ± 9.0	131.1 ± 46.4	<0.0001*
Triglicéridos, mg/dl,	117.8 ±71.0	95.4 ±36.1	138.0 ± 87.3	<0.0001*
Colesterol, mg/dl,	165.8 ± 44.2	180.3 ±38.9	151.1 ±40.3	<0.0001*
IMC, kg/m^2,	24.4 ±2.8	23.5 ± 2.7	25.9 ±2.5	0.06
Linfocitos, x10^3 µl,	2.1 ±0. 9	2.1 ± 0.8	2.1 ± 0.9	0.70
Neutrófilos, x10^3 µl,	4.7 ± 2.1	4.3 ± 1.9	5.0 ± 2.1	0.02*
Paquetas, x10^3 µl,	283.0 ± 116.0	279.2 ± 111.8	286.1 ± 120.1	0.70
Mujeres, N (%)	67 (41.9%)	37 (46.8%)	30 (37%)	0.21
Debut clínico del CC, N (%)				
Subclínico	146 (91.2%)	74 (93.7%)	72 (88.9%)	0.28
Agudo	14 (8.8%)	5 (6.3%)	9 (11.1%)	
ECOG, N (%)				
0	99 (61.9%)	56 (70.9%)	43 (53.1%)	0.02*
≥ 1	61 (38.1%)	23 (29.1%)	38 (46.9%)	
CEA (ng/mL), N (%)				
≤5	87 (54.4%)	47 (82.5%)	40 (75.5%)	0.39
>5	23 (14.4%)	10 (17.5%)	13 (24.5%)	
N/A	50 (31.2%)	-	-	
pT, N (%)				
T1-T2	53 (33.1%)	24 (30.4%)	29 (35.8%)	0.61
T3-T4	107 (66.9%)	55 (69.6%)	52 (64.2%)	
pN, N (%)				
N0	101 (63.1%)	48 (60.8%)	53 (65.4%)	0.54
N+	59 (36.9%)	31 (39.2%)	28 (34.6%)	
Grado, N (%)				
Low grade	147 (91.9%)	72 (91.1%)	75 (92.6%)	0.73
High grade	13 (8.1%)	7 (8.9%)	6 (7.4%)	
Terapia adyuvante, N (%)	54 (33.8%)	29 (36.7%)	25 (30.9%)	0.43
Localización del tumor, N (%)				
Right	75 (46.9%)	32 (40.5%)	43 (53.1%)	0.11
Left	85 (53.1%)	47 (59.5%)	38 (46.9%)	
Invasión linfovascular, N (%)				
Yes	23 (14.4%)	12 (18.8%)	11 (16.4%)	0.72
No	108 (67.5%)	52 (81.2%)	56 (83.6%)	
N/A	29 (18.1%)	-	-	
Estadío, N (%)				
Low	100 (62.5%)	49 (62.0%)	51 (63.0%)	0.90
High	60 (37.5%)	30 (38.0%)	30 (37.0%)	
Recurrencia, N (%)	16 (10.0%)	7 (8.9%)	9 (11.1 %)	0.63
Muerte, N (%)	23 (14.4%)	11 (13.9%)	12 (14.8%)	0.87
Muertes relacionadas con cáncer, N (%**)	12 (52.1%)	5 (45.4%)	7 (58.3%)	0.53

Tabla 6. Características clinicopatológicas de los pacientes diabéticos y no diabéticos con cáncer de colon. Datos expresados como media ± SD o N (%).

* p<0.05 denota significación estadística.
** % de muertes totales
Abreviaturas: IMC, Índice de masa corporal; ECOG, Eastern Cooperative Oncology Group; CEA, antgígeno carcinoembrionario; SD, desviación estándar; N/A, No aplica

2.4.3. Influencia del ambiente diabético en el crecimiento tumoral de los xenoinjertos en los ratones con diabetes inducida con estreptozotocina

Una vez concluido que los datos clínicos no apoyaban ninguna relación entre la DM y las características específicas del tumor, se analizó en los ratones la influencia de la DM en el crecimiento y en las características histológicas de los xenoinjertos de CC humano, siguiendo el protocolo experimental representado en la figura 5.

Los ratones con STZ-D mostraron unas cifras de glucosa de media superiores a 200 mg/ml durante los días que duró el experimento. Al finalizar el seguimiento no se encontraron diferencias significativas en el volumen tumoral entre los ratones control y los diabéticos (figuras 6a).

El análisis morfológico del tumor en el grupo control y en el diabético revelaron unas características arquitectónicas y citológicas idénticas, mostrando un homogéneo patrón de crecimiento sólido con aisladas estructuras glandulares diferenciadas, bordes invasivos hacia los tejidos blandos circundantes y focos de necrosis intratumoral. De forma similar, no se encontraron diferencias en las tasas de proliferación (figura 6b, 6c). El examen de los distintos órganos no mostró diseminación de las células tumorales.

El análisis de la densidad microvascular en el tumor utilizando CD31 como marcador endotelial para la cuantificación mostró similares resultados en el grupo control y en el diabético inducido con estreptozotocina (figura 7).

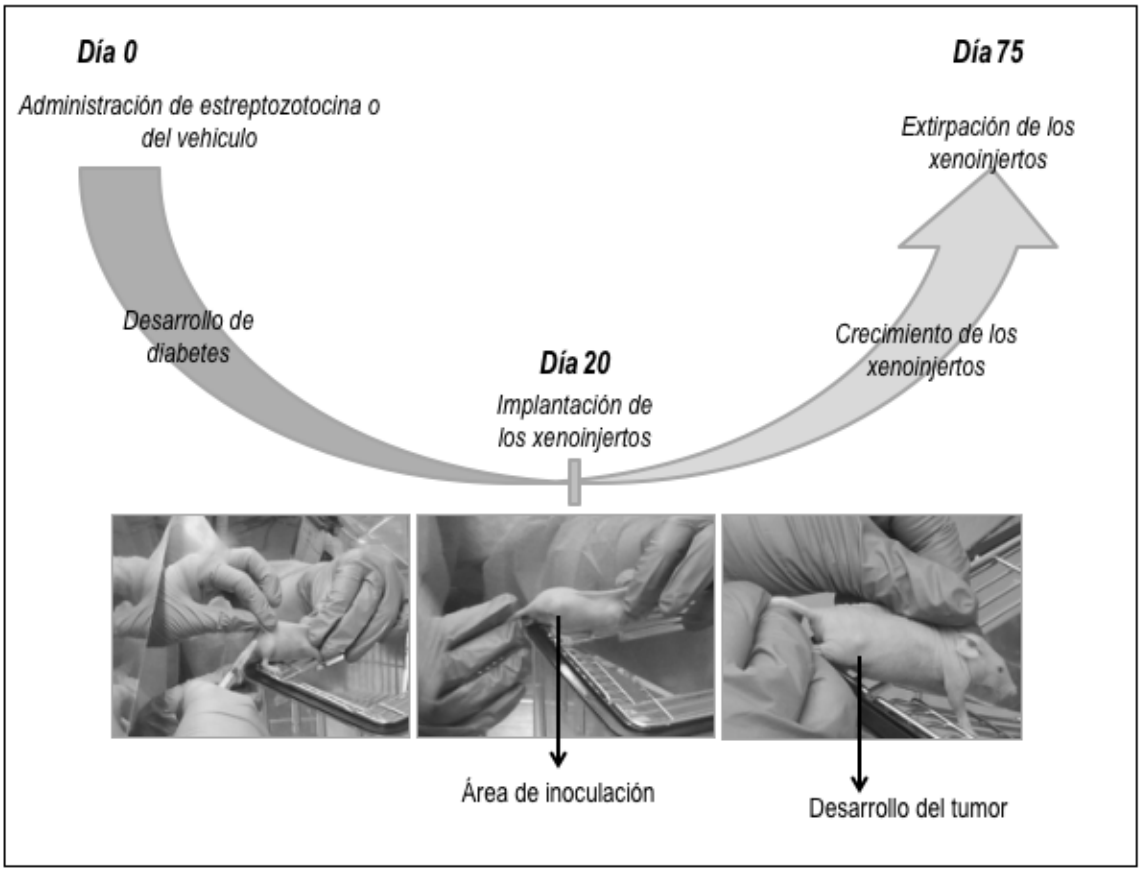

Fig. 5. Procedimiento llevado a cabo para el desarrollo de los xenoinjertos.

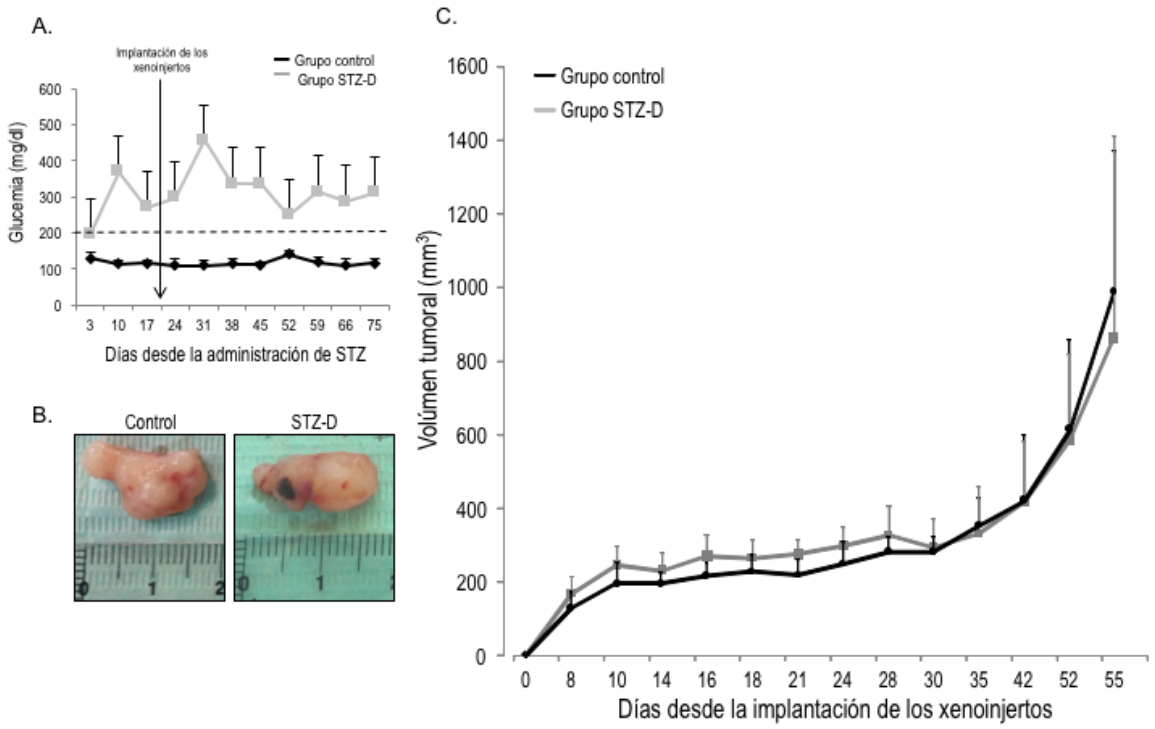

Fig. 6. Diferencias entre glucemias, tamaño y volumen tumoral entre ratones diabéticos y controles.

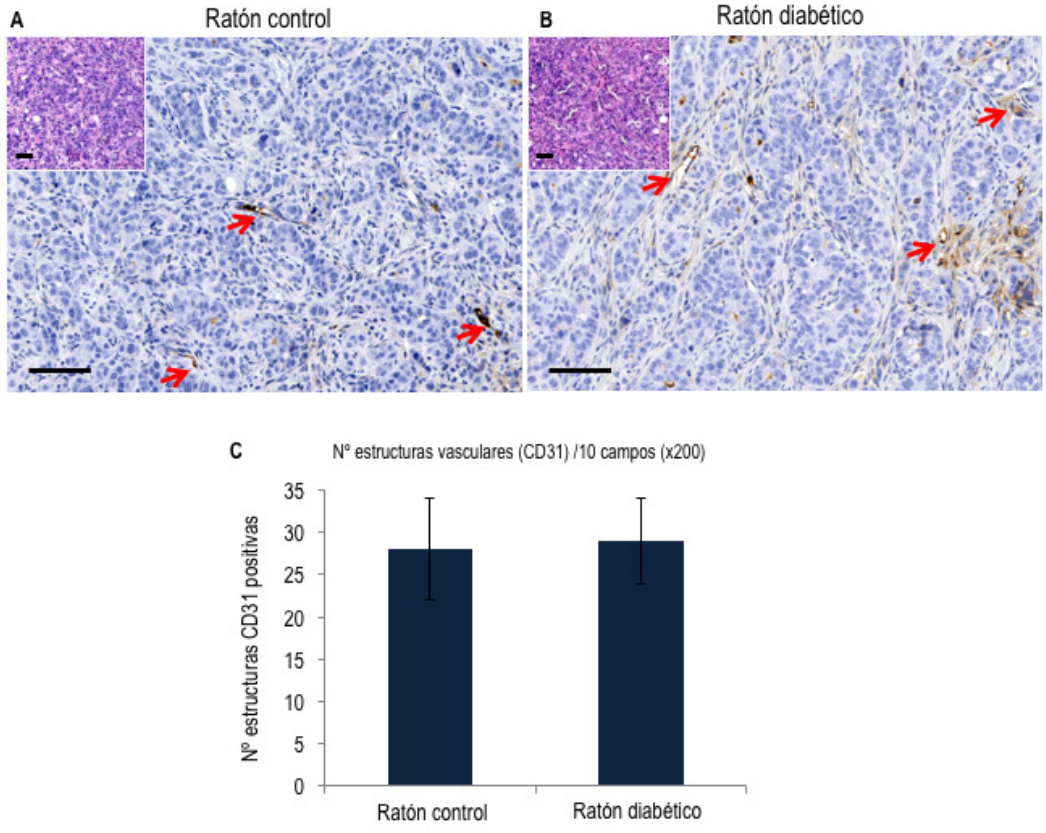

Fig. 7. Diferencias inmunohistoquímicas entre ratones diabéticos y controles.

2.6. Discusión

Existen muchos estudios epidemiológicos que evalúan el riesgo de desarrollar cáncer en pacientes diabéticos, aunque son pocos los que se han centrado en la influencia de la DM en el comportamiento del CC una vez que ha sido ya desarrollado. Este trabajo muestra que no existen diferencias en el comportamiento tumoral o en las características del CC en los pacientes diabéticos comparados con los no diabéticos, o en xenoinjertos de CC implantados en ratones diabéticos o ratones control.

La principal fortaleza de este estudio es la homogeneidad, el correcto balanceo y la caracterización de la población del estudio clínico, lo que limita los sesgos que aparecen en estudios más heterogéneos o en poblaciones peor caracterizadas [176,183,184]. Otra fortaleza es el estudio preclínico que apoya las conclusiones obtenidas tras analizar los datos clínicos.

Los resultados de este trabajo están en concordancia con los del grupo Europeo Americano, étnicamente cercano a esta cohorte [176]. Por lo tanto, no existen diferencias entre diabéticos y controles en términos de características anatómicas o patológicas como estadio o localización izquierda/derecha. En el estudio publicado con anterioridad por Sharma y col. en el que encuentran asociación entre la DM y características histopatológicas peores presenta limitaciones debido a la heterogeneidad de los pacientes entre los que se incluyen histologías diversas, como carcinoma microcítico de recto, y carcinomas de la misma zona anatómica [184]. El cáncer de colon y el cáncer rectal muestran diferencias biológicas y clínicas probablemente relacionadas con mecanismos diferentes de carcinogénesis [182]. Incluso el tratamiento estándar del cáncer de recto muestra más variabilidad entre los distintos centros oncológicos dependiendo de la experiencia y habilidad de los equipos multidisciplinares. En este estudio, el carcinoma de recto ha sido excluido ya que su tratamiento estándar incluye quimioradioterapia adyuvante con el fin de preservar la estructura anatómica esfinteriana y conseguir los mejores resultados. Este tratamiento modifica esencialmente la integridad celular y su microambiente e incluso en muchas ocasiones consigue la desaparición completa del tumor, siendo en ese caso imposible realizar los análisis requeridos para estudios como este. Por lo tanto, en esta serie y en cualquier otra con estos objetivos, el carcinoma rectal debería de analizarse a parte. Por último, el adenocarcinoma es el subtipo histológico asociado con el ambiente pro-inflamatorio. Por lo tanto, si lo que queremos estudiar en la contribución al mecanismo de carcinogénesis, otras histologías deberían de ser excluidas.

Muchas publicaciones sugieren que la medicación utilizada para el control de las cifras de glucosa, principalmente la metformina, podría modificar el riesgo de desarrollar un cáncer [153,185]. El consumo de metformina se ha visto asociado a un descenso del riesgo de CCR y un aumento de la supervivencia [151,186,187,188]. Una revisión sistemática de 12 ensayos randomizados (21.595 pacientes) y 41 estudios observacionales (1.029.389 pacientes) encontró que en los estudios observacionales, el riesgo de CCR era 17% más bajo en pacientes diabéticos tratados con metformina que en aquellos tratados con otros agentes [189]. Otros meta-análisis han confirmado este papel protector asociado a la metformina comparado con otros agentes hipoglicemiantes o con la insulina. En un meta-análisis de 21 estudios observacionales, la metformina estaba relacionada con un descenso de la mortalidad cáncer-específica (HR 0,74, 95% CI 0,62-0,88). El análisis por subgrupos de cáncer mostró una reducción significativa en la mortalidad asociada a CC (4 estudios, HR 0,65; 0,56-0,76) pero no a cáncer de mama o de próstata [190]. En los pacientes con CCR que toman metformina se estima que el beneficio en la supervivencia global es de un 44% comparado con los que no la consumen [151]. Son varios los mecanismos posibles para este efecto antitumoral. La metformina reduce la insulina circulante, promueve la pérdida de peso [191] y activa la protein-quinasa 5´adenosina monofosfato activada, inhibiendo el crecimiento de células de cáncer de colon [192]. La metformina, sola o en combinación con oxaliplatino, reduce la agresividad de tumores colorrectales en ratones diabéticos [193]. Publicaciones más anteriores ya describían el aumento de expresión de la glucosa-fosfato deshidrogenasa mitocondrial, diana de la metformina, en tumores indiferen-

ciados, de rápido crecimiento, pero no en los de crecimiento lento [194]. En este sentido, la mayoría de las células malignas son altamente glicolíticas y producen altos niveles de especies reactivas de oxígeno comparadas con las células normales. Sin embargo, no existen datos sobre la expresión de la glucosa-fosfato deshidrogenasa mitocondrial en células de CCR [195]. En pacientes no diabéticos, la toma de metformina en dosis bajas durante periodos no prolongados reprime el desarrollo de focos aberrantes en las criptas colorrectales [196]. En un ensayo randomizado fase III, multicéntrico y doble ciego, a administración de bajas dosis de metformina (250 mgr) durante un año en pacientes no diabéticos demostró ser segura y redujo la prevalencia y el número de adenomas o pólipos después de la polipeptomía [197].

A la vista de estas afirmaciones de la literatura, el subgrupo de pacientes que toma metformina ha sido analizado por separado en esta cohorte. Tampoco en este grupo se ha observado ningún efecto de éste fármaco sobre el comportamiento del tumor. Sin embargo, estos resultados deben de ser interpretados con prudencia ya que el número de casos es escaso. Aún así y a pesar de este escaso número, nuestros resultados concuerdan con múltiples publicaciones que demuestran que la metformina puede reducir en riesgo de cáncer, aunque no está claro su efecto sobre la mortalidad asociada a un cáncer ya establecido [198,199], incluso en otros tipos de tumor [200,201].

El modelo preclínico in vivo está basado en un modelo publicado [202] y extensamente aceptado, tratándose de una aproximación experimental homogénea y reproducible con resultados robustos y sin sesgos. Este procedimiento experimental no revela diferencias estadísticamente significativas en la cinética del crecimiento tumoral ni en las características histopatológicas existentes en los ratones diabéticos y controles. Muy pocos estudio in vivo han estudiado la influencia de la DM en el crecimiento de un tumor humano. Un estudio no observó diferencias en la cinética de un sarcoma xenoinjertado en ratones diabéticos y controles [203]. Como contraste, un estudio experimental similar muestra un efecto protector de la DM en xenoinjertos de cáncer de próstata [181]. Sin embargo, una cohorte publicada de pacientes con cáncer de próstata que estudia el efecto de la DM en este tipo de cáncer mostró resultados heterogéneos [204].

La debilidad de este estudio en su parte epidemiológica y clínica es la naturaleza retrospectiva del mismo y el relativo pequeño tamaño de la muestra, que pertenece a un único centro. El reducido tamaño de la muestra ha permitido el estudio en detalle de la subpoblación, tarea imposible de hacer con grandes muestras, aunque la procedencia de un único centro limita la extrapolación de los resultados a otros centros o a otros países.

Este trabajo sirve como base para el diseño de estudios más amplios, multicéntricos y, esperamos, confirmatorios.

2.7. Conclusiones

- No existen diferencias histopatológicas en las muestra de pacientes con CCR analizada que sean atribuibles al efecto de la diabetes.
- No existen diferencias histopatológicas ni en la cinética celular entre los ratones xenoinjertados diabéticos y no diabéticos estudiados.
- La diabetes mellitus produce un efecto leve o incluso insignificante sobre el comportamiento y las características de un cáncer de colon ya establecido.

4. Apéndice

Información adicional

La realización de este trabajo ha sido financiada por el Proyecto integrado de excelencia en institutos de investigación sanitaria acreditados de la convocatoria 2013 de la Acción Estratégica de Salud 2013-2016 (Instituto de Salud Carlos III-Fondos FEDER, referencia:PIE13/00051).

Los resultados presentados en esta tesis han sido publicados parcialmente en los documentos que se adjuntan:

– González N, Prieto I, Del Puerto-Nevado L, Portal-Nuñez S, Ardura JA, Corton M, Fernández-Fernández B, Aguilera O, Gomez-Guerrero C, Mas S, Moreno JA, Ruiz-Ortega M, Sanz AB, Sanchez-Niño MD, Rojo F, Vivanco F, Esbrit P, Ayuso C, Alvarez-Llamas G, Egido J, García-Foncillas J, Ortiz A, Consortium DC. 2017 update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications. Oncotarget. 2017 Jan 3. doi: 10.18632/oncotarget.14472.

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ARTÍCULOS RELACIONADOS CON LA LÍNEA DE INVESTIGACIÓN PUBLICADOS EN REVISTAS INTERNACIONALES

2017 update on the relationship between diabetes and colorectal cancer: epidemiology, potential molecular mechanisms and therapeutic implications

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ABSTRACT

Worldwide deaths from diabetes mellitus (DM) and colorectal cancer increased by 90% and 57%, respectively, over the past 20 years. The risk of colorectal cancer was estimated to be 27% higher in patients with type 2 DM than in non-diabetic controls. However, there are potential confounders, information from lower income countries is scarce, across the globe there is no correlation between DM prevalence and colorectal cancer incidence and the association has evolved over time, suggesting the impact of additional environmental factors. The clinical relevance of these associations depends on understanding the mechanism involved. Although evidence is limited, insulin use has been associated with increased and metformin with decreased incidence of colorectal cancer. In addition, colorectal cancer shares some cellular and molecular pathways with diabetes target organ damage, exemplified by diabetic kidney disease. These include epithelial cell injury, activation of inflammation and Wnt/β-catenin pathways and iron homeostasis defects, among others. Indeed, some drugs have undergone clinical trials for both cancer and diabetic kidney disease. Genome-wide association studies have identified diabetes-associated genes (e.g. *TCF7L2*) that may also contribute to colorectal cancer. We review the epidemiological evidence, potential pathophysiological mechanisms and therapeutic implications of the association between DM and colorectal cancer. Further studies should clarify the worldwide association between DM and colorectal cancer, strengthen the biological plausibility

of a cause-and-effect relationship through characterization of the molecular pathways involved, search for specific molecular signatures of colorectal cancer under diabetic conditions, and eventually explore DM-specific strategies to prevent or treat colorectal cancer.

BACKGROUND

Diabetes mellitus (DM) and cancer are among the most frequent causes of death worldwide. According to Global Burden of Disease data, from 1990 to 2013 mortality from DM increased by 90% [1]. Colorectal cancer (CRC) is among the top causes of cancer death. From 1990 to 2013 global deaths from CRC increased by 57%, [1]. In the United States, CRC is the second leading cause of cancer death in men and women combined (http://www.ccalliance.org/colorectal_cancer/statistics.html) [2]. A link between DM and cancer is now recognized in American Diabetes Association (ADA) guidelines, following a 2010 consensus report [3, 4]. If the association holds, the current worldwide diabetes epidemic, fueled by life-style changes, may trigger a wave of CRC diagnoses. However, this knowledge has had limited impact on clinical care in the form of specific diagnostic tests or therapeutic approaches supported by clinical guidelines. Furthermore, on a worldwide basis the prevalence of DM and the incidence of CRC are not correlated, suggesting that country-specific factors may play a role in the association between DM and CRC (Figure 1). Annual CRC incidence rates vary more than ten-fold worldwide, the highest rates being in developed countries such as Korea (age-standardized rate 45 per 100, 000), Australia and Ireland, and the lowest in Western Africa (e.g. Cameroon 3.3 per 100, 000) (http://globocan.iarc.fr). By contrast, DM prevalence is highest in Egypt and United Arab Emirates (20, 000 per 100, 000, and lowest in Australia (5, 100), Ireland (4, 400) and Western Africa (www.diabetesatlas.org/). A better understanding of the factors underlying regional differences may provide clues to the relationship between DM and CRC. We now review the epidemiological evidence, potential pathophysiological mechanisms and therapeutic implications of the association between DM and CRC and propose a research agenda that may impact clinical practice to prevent or treat CRC in DM patients. A Pubmed search with the key words "(diabetes OR insulin OR hyperglycemia) AND (colon OR colorectal) AND cancer" was performed with no time cut-off points and further references added from the reference list of the publications found or based on the authors own experience knowledge.

DIABETES MELLITUS

DM is characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. Chronic hyperglycemia is associated with injury to the kidneys, heart, nerves, eyes and blood vessels [5]. In type 1 DM (T1DM, 5-10% of DM cases), cell-mediated autoimmune destruction of pancreatic β-cells causes absolute insulin deficiency. Type 2 DM (T2DM) is characterized by insulin resistance and relative insulin deficiency. T2DM patients are frequently obese and older at DM onset than T1DM patients [6]. Obesity promotes insulin-resistance and is thought to be a major driver of the current DM epidemic. Mendelian-inherited genetic defects of β-cells or of the insulin signaling machinery also cause DM [5].

Mean age at diagnosis of DM is 54 years in the US (http://www.cdc.gov/diabetes/statistics/age/). Therapies for DM increase insulin availability (insulin or insulin analog administration or agents that promote insulin secretion), improve sens itivity to insulin, decrease glucose synthesis, delay the gut absorption of carbohydrate, or increase urinary glucose excretion (Supplementary Table 1). The preferred initial and most widely used pharmacological agent for T2DM is metformin, which decreases glucose production by inhibiting the mitochondrial glycerophosphate dehydrogenase (GPDH, GPD2) [7]. If adequate glucose control is not achieved within 3-6 months, a second oral agent, a Glucagon-like peptide-1 (GLP-1) receptor agonist, or insulin should be added [8, 9].

COLORECTAL CANCER

CRC originates from colon epithelium [10]. Over 70% CRCs are sporadic, resulting from dietary and environmental factors. The incidence increases with age and they usually occur over the age of 50 years. True inheritable CRC (<10% of cases) may be associated or not to colonic polyps (Table 1) [11]. The familial type (25% of cases) is associated with a family history of CRC or large adenomas, in the absence of classic Mendelian inheritance [12]. Right- and left-sided CRCs exhibit different epidemiological patterns, sensitivities to chemotherapy and outcomes, probably related to different molecular characteristics and chromosomal instability with left-sided tumors [13].

CRC is initiated by mutations in tumor suppressor genes (adenomatous polyposis coli or *APC*, *CTNNB1, p53*) and oncogenes (*KRAS*). Accumulation of multiple mutations leads to a selective growth advantage for transformed epithelial cells that is modulated by epigenetic changes [14, 15]. Diet, the microbiota and the inflammatory response to the microbiota are potential players [16-22]. Indeed, chronic gut inflammation (e.g.

Table 1: Genetics of colorectal cancer and potential impact of DM on colorectal cancer-related genes

Colorectal cancer	Mutation	Inheritance	Impact of DM on gene expression *	Reference
Familial adenomatous polyposis	Inactivating germline mutation in adenomatous polyposis coli (*APC*)	Autosomal dominant	Increased *APC*	[283,284]
MUTYH-associated polyposis	Inactivating germline mutation in *MUTYH*	Autosomal recessive	Unchanged *MUTYH*	[283,284]
Peutz-Jeghers syndrome	Inactivating germline mutation in serine threonine kinase 11 (*STK11*)	Autosomal dominant	Increased *STK11*	[285]
Hereditary non-polyposis colorectal cancer (Lynch syndrome)	Inactivating germline mutation in *MLH1, MSH2, MSH6*, or *PMS2*	Autosomal dominant	Unchanged *MLH1, PMS2* Increased *MSH2, MSH6*	[286]
Chromosomal instability (frequent)	Acquired accumulation of numerical (aneuploidy) or structural chromosomal abnormalities and mutations in specific oncogenes and tumor suppressor genes (e.g. *APC, PIK3CA, SMAD4, KRAS, TP53, BRAF*)		Unchanged *PIK3CA, SMAD4, BRAF* Increased *KRAS, TP53*	[287–289]

* Kidney gene expression in human diabetic kidney disease transcriptomics (http://www.nephromine.org).

Table 2: Key risk factors for T2DM, colorectal cancer and DM complications (Diabetic kidney disease)

Risk factor	T2DM	Colorectal cancer	Diabetic kidney disease
Race	African American, Native American	African American	African American, Native American
Obesity	Yes	Yes	Yes
Inflammation	Yes	Yes	Yes
Microbiota	Yes	Yes	Unknown
Low vitamin D	Yes	Yes	Yes
High protein (meat protein) diet	Yes	Yes	Yes
Low fiber diet	Yes	Yes	ND
No Mediterranean diet	Yes	Yes	ND
Low magnesium intake/ hypomagnesemia	Yes	Yes	Yes
Angiotensin II	Yes	Yes	Yes
Age	Yes	Yes	Unclear

ulcerative colitis or Crohn´s disease) is associated with increased incidence of colon cancer. A major molecular pathway is Wnt signaling activation of the transcription factor β-catenin to promote expression of cell proliferation genes. Loss-of-function mutations or epigenetic silencing of *APC* leads to aberrant β-catenin accumulation and uncontrolled cell proliferation. The normal APC protein forms a complex with glycogen synthase kinase 3-beta (GSK-3β) that allows GSK-3β to phosphorylate β-catenin, targeting it for ubiquitination and proteasomal degradation, thus decreasing β-catenin-dependent transcriptional events [23].

Early-stage CRC is treated with surgery and locally advanced CRC (radically resected stage III and 'high-risk' stage II disease) with adjuvant chemotherapy on top of surgery. Rectal cancer with nodal disease standard treatment includes neoadjuvant chemo-radiation [24]. Adjuvant chemotherapy schemes contain 5-fluorouracil and oxaliplatin. Metastatic CRC is treated with irinotecan or oxaliplatin combined with a fluoropyrimidine and leucovorin (FOLFIRI or FOLFOX regimens) [25]. Addition of targeted therapies over the past 10 years has improved overall survival. Testing for KRAS, NRAS, BRAF, PIK3CA and PTEN mutations is used to assess the potential clinical benefit of anti-Epidermal Growth Factor Receptor (anti-EGFR) and panitumumab treatment. Meta-analyses suggest that mutation testing for KRAS exon 2 is the strongest biomarker of response. The addition of anti-Vascular Endothelial Growth Factor (anti-VEGF) agents (bevacizumab, regorafenib) to chemotherapy of metastatic CRC prolongs progression-free and overall survival in first- and second line therapy [26].

EPIDEMIOLOGICAL ASSOCIATION BETWEEN DIABETES AND CRC

Epidemiological studies suggest that DM, especially T2DM, is associated with increased risk of cancer at several sites, including CRC [27] (Table 3). The first prospective association was reported in 1998 in US participants followed from 1960 to 1972 [28]. The adjusted incidence density ratio of CRC was 1.30 (95%

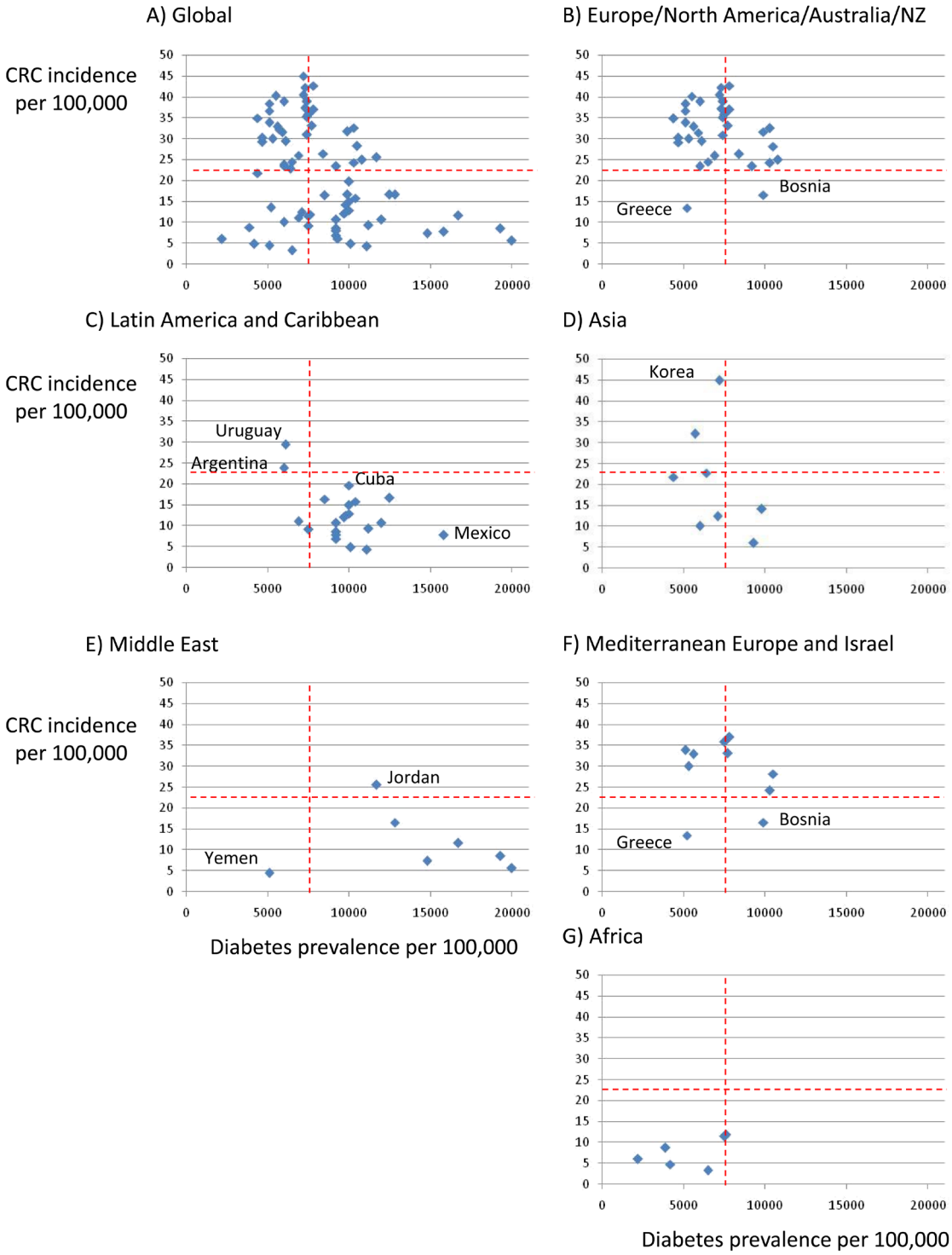

Figure 1: Relationship between incidence of colorectal cancer (CRC) and prevalence of DM in different parts of the world. A. Global, **B.** Europe, North America and Australia/New Zealand, **C.** Latin America and Caribbean, **D.** Asia, **E.** Middle East, **F.** European Mediterranean countries and Israel, **G.** Africa. IDF 2015 data for DM (www.diabetesatlas.org/) and Globocan 2012 data for colorectal cancer (http://globocan.iarc.fr/Pages/age-specific_table_sel.aspx). Discontinuous red lines represent median values for the global population. Regional differences can be identified by the location of countries within the four quadrants. Note regional differences as well as countries that differ from others in the region. Regions are more clearly separated by CRC incidence than by DM prevalence, Europe/ North America/Australia/NZ is the only high DM/high CRC region. Latin America and Caribbean is a high DM/low CRC region with the exception of Argentina and Uruguay where meat intake is high, while in the opposite extreme Mexico a is very high DM/low CRC country. In the Middle East a high prevalence of DM is not associated with high CRC incidence, unlike in European Mediterranean countries which in general behave as the rest of Europe. Korea is an example of low DM/high CRC country in Asia.

confidence interval (CI) 1.03-1.65) for diabetic males, but not significant for females. The association was found only among non-smoker males. A more recent prospective US study followed an older cohort from 1995 to 2004 and observed an increased adjusted Hazard Ratio (HR) for CRC in both males and females [29]. Lifestyle changes from the 60s to the 90s may explain the change in female risk. A similar association has been reported in Japan [30], China [31], Australia [32] or certain European countries (e.g. Sweden) [33], among others. A recent umbrella review of meta-analyses of observational studies on T2DM and cancer updated to the end of 2013 concluded that CRC was one of only four cancer sites associated to T2DM with robust supporting evidence and without hints of bias [34]. Furthermore, in a meta-analysis of prospective cohort studies encompassing near a million participants, prediabetes (impaired fasting glucose and/or impaired glucose tolerance) was also associated with increased risk of CRC [35]. However, uncertainties remain. The presence of detection bias and/or reverse causation has been suggested by studies in Australia, Israel and the Netherlands that found a higher risk of cancer within 3 months of a DM diagnosis [32, 36, 37]. In this regard, in the US, respondents with diabetes were 22% more likely to be up-to-date on CRC screening than those without diabetes [38]. A higher risk of developing DM within 5 years of CRC diagnosis was also reported [39]. In addition, regional differences exist: in Norway and the Netherlands only diabetic females had a higher incidence of proximal colon cancer or CRC [40, 41], while no association was found in Tyrol. Unraveling the reasons underlying regional differences may provide clues to the association and to public health interventions. Potential differences in the use of specific antidiabetic drugs may play a role as discussed below. Furthermore, epidemiological data from developing countries are scarce. This is an important piece of missing information since almost 55% of CRC cases occur in more developed regions (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx), while 80% of DM patients live in low- and middle-income countries (www.diabetesatlas.org/).

Risk factors shared by CRC, DM, and DM target organ damage may be confounders in epidemiological studies (Table 2). Obesity is a major risk factor for T2DM, cancer and diabetic kidney disease (DKD) [42, 43]. However, key studies observing an association between DM and CRC were adjusted by BMI. In this regard, there may be a relationship between obesity, insulin resistance and CRC. In a prospective European study, lower CRC risk was observed for metabolically healthy/overweight individuals compared with metabolically unhealthy/overweight individuals, defined as individuals with higher C-peptide levels indicative of hyperinsulinaemia [44]. Diet may be another confounder. A high meat intake increases and a Mediterranean diet decreases both the risk of DM and of CRC [45].

POTENTIAL MOLECULAR MECHANISMS OF THE ASSOCIATION BETWEEN DM AND CRC

The association between DM and CRC may result from shared risk factors between T2DM and cancer but epidemiological data suggest a potential contribution of hyperinsulinemia, hyperglycemia or DM therapy [4, 46, 47] (Figure 2). Additionally, the DM microenvironment, such as advanced glycation end-products (AGEs),

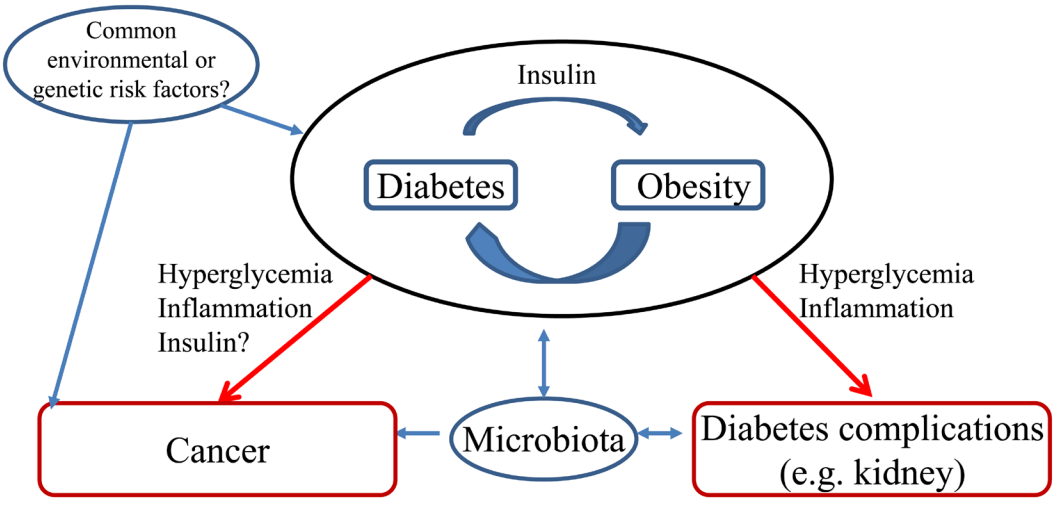

Figure 2: Hypotheses potentially explaining the association between diabetes and colorectal cancer. Two major potential relationships have been depicted. **A.** Common risk factors (e.g. diet, genetic) favor both diabetes and colorectal cancer; **B.** Diabetes favors cancer development. These potential relationships are put in context with the occurrence of other diabetes complications such a chronic kidney disease. Obesity is a known risk factor for both colorectal cancer and diabetic kidney disease.

hyperlipidemia, local inflammation/oxidative stress, extracellular matrix alterations, and altered microbiota or ischemia due to vasculopathy may recruit secondary mediators of injury that may favor the development of both cancer and other complications of DM such as DKD.

Insulin

Insulin and insulin-like growth factor (IGF)-1 have growth factor and antiapoptotic properties in a variety of cultured tumor and non-tumor cell types, including normal colon epithelium and colon cancer cells [48, 49]. These actions have been interpreted as part of a putative tumor-enhancing effect of insulin [50, 51]. However, insulin signaling is also required for survival and function of healthy cells in vivo, such as podocytes, key cells in DKD, and selective podocyte insulin resistance reproduces features of DKD in the absence of hyperglycemia [52]. The mTOR and p21-activated protein kinase-1 (PAK-1)/Wnt/β-catenin intracellular pathways are involved in insulin-stimulated proto-oncogene expression in intestinal cells [53]. These molecular pathways also mediate diabetic complications, including DKD [54].

An increased incidence of azoxymethane-induced intestinal tract cancer was observed in preclinical models of obesity and T2DM, including obese Zucker rats and KK Ay, db/db and ob/ob mice [55-57]. The addition, the incidence and multiplicity of intestinal adenomas was higher in db/db mice with Apc mutations than in non-diabetic mice [58]. However, the relative role of hyperglycemia, hyperinsulinemia or obesity was not characterized.

The role of hyperinsulinemia was studied in a normoglycemic model of mammary cancer growth, but results do not necessarily extrapolate to CRC [59]. A tyrosine kinase inhibitor specific to the insulin and IGF-1 receptors aggravated hyperinsulinemia but prevented insulin signaling and cancer growth. However, tyrosine kinase inhibitors are promiscuous and are in clinical use as anti-tumor agents. Thus, the fact that members of an anti-tumor agent family decrease tumor growth is not definitive evidence for a role of insulin. CL-316243, a β3-adrenergic receptor agonist that sensitizes to insulin action, reduced hyperinsulinemia and phosphorylation of insulin and IGF-1 receptors and attenuated mammary tumor progression, supporting a role for hyperinsulinemia in T2DM associated tumor progression [60].

Hyperglycemia

Hyperglycemia has been implicated both in colon cancer growth and in DKD and some of the molecular mechanisms are shared by both diseases. High glucose levels and AGEs increase proliferation and migration of cultured colon cancer cells [61, 62]. High glucose levels also enhance resistance to 5-fluoruracil-induced apoptosis [63]. AGE-induced CRC cell proliferation requires carbohydrate response element-binding protein (ChREBP) [64], a key transcription factor also involved in DKD [65]. The polyol and hexosamine pathways, which increase glucose oxidation, are upregulated in diabetes target organ epithelial cells [54] and in colon cancer [66]. Hyperglycemia and AGEs induce oxidative stress and inflammation, which can damage cellular components and contribute to malignant cell transformation [67-69]. High glucose-induced oxidative stress plays a pivotal role in the development of diabetes complications by activating different pathways, such as the transcription factor nuclear factor-kappa B (NF-κB) [70, 71]. Indeed, bardoxolone methyl, a potent nuclear factor erythroid 2-related factor 2 (Nrf2) activator/NF-κB inhibitor, improved glomerular filtration in RCT in DKD [72]. Interestingly, the observation that bardoxolone increased glomerular filtration was first made in clinical trials exploring its anticancer potential.

The Warburg effect refers to the high glucose uptake and metabolism of glucose through glycolysis rather than aerobic phosphorylation in tumor cells despite the presence of oxygen [73, 74]. Glycolysis is less efficient but generates adenosine triphosphate (ATP) faster, conferring a growth advantage to tumor cells. Upregulation of insulin-independent glucose transporters such as glucotransporter-1 (Glut-1) favors glucose uptake by cancer cells [75, 76]. Glut overexpression is usually translated into higher proliferation rates. The diabetic milieu and transforming growth factor (TGF)-β1 upregulate renal cell Glut-1 and this is thought to contribute to the pathogenesis of DKD [77].

Few preclinical studies have addressed the impact of hyperglycemia *per se* (i.e. T1DM) on colon cancer. Streptozotocin-induced hyperglycemia, an insulin-deficiency DM model, increased liver metastasis of mouse colon cancer cells, while glycemic control with either insulin or gliclazide was protective [78]. These studies suggest that hyperglycemia per se may favor colorectal tumor growth and that hyperglycemia may be a more powerful stimulus for tumorigenesis than insulin in experimental animals.

Wnt/β-catenin is activated in CRC as a direct consequence of *APC* mutations and in kidney cells in DKD [79], protecting glomerular mesangial cells from high-glucose-mediated cell apoptosis [80] but causing podocyte dysfunction and proteinuria [79]. β-catenin expression and altered phosphorylation, and cell proliferation were higher in normal colon epithelium surrounding tumor tissue in diabetic than in non-diabetic patients [81]. VDR activation antagonizes Wnt/β-catenin signaling [82] (Figure 3). The nephroprotective action of VDR activators has been related to Wnt/β-catenin inhibition [83]. Vitamin D deficiency is common in DM [84] and has also been associated with increased risk of CRC [85, 86]. High-

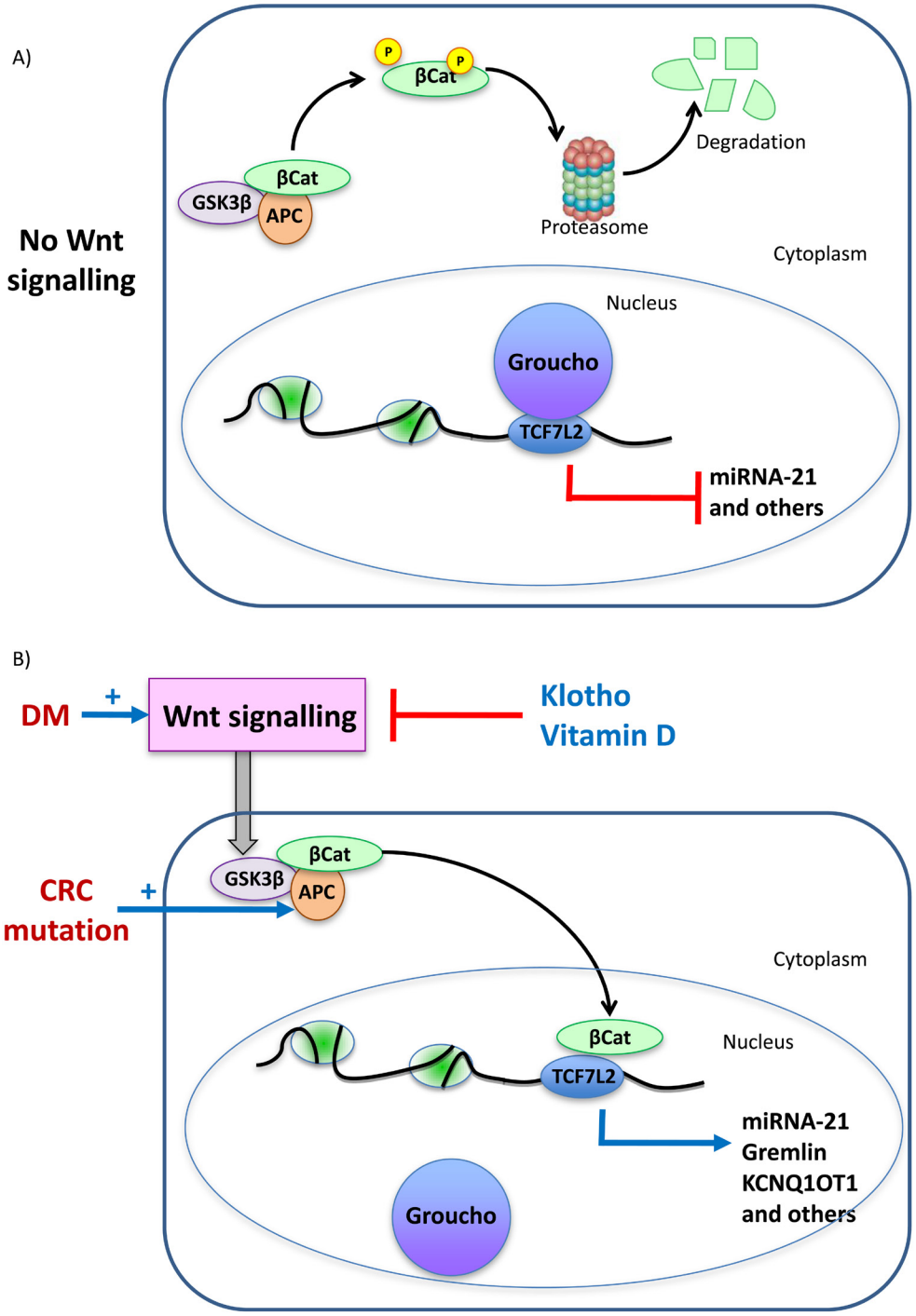

Figure 3: Key molecular pathways potentially linking diabetes and colorectal cancer. The example of β-catenin activation. **A.** In the absence of Wnt signaling, APC-bound glycogen synthase kinase 3-beta (GSK-3β) phosphorylates β-catenin (βCat), targeting it for ubiquitination and proteasomal degradation. In the absence of nuclear β-catenin, Groucho binds to transcription factors of the TCF family, repressing transcription. The TCF family includes TCF7L2 which has been associated to DM, DM complications and colon cancer by GWAS studies. **B.** Colon cancer is characterized by loss of function mutations of APC and in DM Wnt signaling is activated. Klotho and vitamin D prevent Wnt signaling and are protective against tumors and against DM complications. Wnt signaling prevents β-catenin phosphorylation and degradation allowing its nuclear migration, where it displaces Groucho and promotes transcription of genes involved in cell proliferation as well as other genes such as miR-21. miR21 contributes to tumorigenesis and to diabetes complications such as kidney injury. GWAS identified a *GREM1* SNP associated with CRC susceptibility that facilitates TCF7L2 binding to DNA, leading to stronger *GREM1* gene expression. A *GREM1* SNP also associate with diabetic kidney disease. The gene product, Gremlin, promotes kidney injury in DM as well as colon cancer cell migration. *KCNQ1* was associated with T2DM by GWAS. This locus encodes KCNQ1OT1, a β-catenin target upregulated in CRC.

Table 3: Epidemiological association between DM and risk of CRC. 95% confidence interval shown.

Country	N (x1000)	Mean age (years)	Period (years)	Location	Males	Females	Overall	Ref
US*	850	54	59-72	CRC	1.30 (1.03-1.65)	1.16 (0.87-1.53)	Not available	[28]
US**	484	62	95-06	CRC	Colon 1.24 (1.12-1.38) Rectum 1.34 (1.14-1.57)	Colon 1.37 (1.16-1.60) Rectum 1.43 (1.08-1.88)	Colon 1.27 (1.17-1.39) Rectum 1.36 (1.18-1.56)	[29]
Japan***	335	N.A.	N.A.	CRC	N.A.	N.A.	1.40 (1.19-1.64)	[30]
China****	327	60	07-13	CRC	Colon 1.47 (1.29-1.67) Rectum 1.25 (1.09-1.43)	Colon 1.33 (1.15-1.54) Rectum 1.29 (1.10-1.51)	Colon 1.40 (1.27-1.55) Rectum1.26 (1.14-1.40)	[31]
Australia****	953	27 (T1DN) 60 (T2DN)	97-08	CRC	1.18 (1.15-1.21)	1.16 (1.13-1.20)	N.A.	[32]
Sweden****	2.9 1.4	N.A.	64-10	CRC	N.A.	N.A.	Colon 1.33 (1.28-1.38) Rectum 1.19 (1.13-1.25)	[33]
Norway***	751 pers/ year	71	84-96	CRC	CRC 0.66 (0.35-1.34)	CRC 1.55 (1.04-2.31) Colon 1.60 (1.02-2.51) Rectum 2.70 (1.29-5.61)	N.A.	[40]
Tyrol****	5.7	58	88-10	CRC	1.11 (0.81-1.49)	0.94 (0.62-1.36)	N.A.	[290]
Israel**	2186	64	02-12	CRC	1.45 (1.37-1.55)	1.48 (1.39-1.57)	N.A.	[36]
Netherlands**	120	62	86-06	CRC	CRC 0.95 (0.75–1.20) Proximal 1.13 (0.76-1.68) Distal 0.77 (0.49–1.21) Rectum 0.50 (0.21–1.22)	CRC 1.08 (0.85–1.37) Proximal 1.44 (1.05–1.99) Distal 0.75 (0.44–1.27) Rectum 1.16 (0.54–2.48)	N.A.	[41]
Meta-analysis***	8244	N.A.	N.A.	CRC	N.A.	N.A.	1.27 (1.21-1.34)	[34]

* Adjusted incidence density ratio: ** Adjusted HR; ***RR. **** Standardized incidence ratios
N.A.: not available

glucose-induced inflammatory and fibrogenic responses in kidney cells contribute to DKD and are prevented by vitamin D receptor (VDR) activation [87-90]

EGFR signaling contributes to tumorigenesis and tumor progression of CRC and EGFR-targeted cetuximab is used to treat CRC. Genetic or pharmacological EGFR blockade slows experimental renal disease progression [91]. High-glucose, AGE, angiotensin II, and pro-inflammatory cytokines, such as TWEAK and parathyroid hormone-related protein (PTHrP) AGE promote EGFR transactivation in kidney cells [92-96]In this regard, TWEAK targeting antibodies are undergoing clinical trials in kidney disease, while targeting the TWEAK receptor Fn14 reduced colon cancer metastasis in experimental animals [95, 97]. Inhibition of EGFR with erlotinib attenuates DKD in experimental T1DM, through inhibition of mTOR [98]. Indeed, mTOR is activated in diabetic podocytes and mTOR targeting protects from DKD [99]. CCN2 is a novel EGFR ligand that promotes kidney inflammation and DKD progression [100, 101] and in CRC cells, regulates cell migration and prevents apoptosis [102].

Klotho is an anti-aging hormone of kidney origin with anti-inflammatory and anti-fibrotic properties [103, 104]. Experimental and human diabetes, inflammation and hyperlipidemia are associated with decreased Klotho expression [105-108]. Loss of Klotho contributes to kidney injury by de-repression of Wnt/β-catenin signaling [109] and similar mechanisms may be active in colon cancer cells. In this regard, Klotho suppresses growth and invasion of colon cancer cells through inhibition of the IGF1R-mediated PI3K/Akt pathway [110] and is frequently inactivated through promoter hypermethylation in CRC [111].

Table 4: Examples of agents in the pipeline targeting both cancer and diabetic target organ complications exemplified by diabetic kidney disease

Activity	Agent	Successful in animal models of cancer	Successful in experimental DKD	RCT in human cancer	RCT in human DKD	Refs
HMGCoA reductase inhibitors	statins	Yes	Yes	Yes	Yes	[253-256]
RAAS targeting drugs	ACE inhibitors, ARBs	Yes	Yes	No	Yes	[252, 257-259]
VDR activator	Paricalcitol	Yes	Yes	Yes	Yes	[265,266,291]
Endothelin receptor antagonists	Atrasentan and others	Yes	Yes	Yes	Yes	[262–264,292–301]
Anti-fibrotic agents	Anti-CTGF mAb FG3019	Yes	Yes	Yes	Yes	[101,302,303]
	Anti- TGF-β1 mAb.	Yes	Yes	Yes	Yes	[304]
Anti-inflammatory agents	Chemokine targeting agents	Yes	Yes	Yes (anti-CXCR4)	Yes (anti-CCL2 and others)	[270]
	JAK/STAT inhibitors	Yes	Yes	Yes	Yes	[276-279]
Inhibitors of epidermal growth factor Receptor/ ligands	Several agents	Yes	Yes	Anti-EGFR antibodies (cetuximab)	Anti-TGF-α/ epiregulin antibody (LY3016859)	[305,306]
mTOR inhibitors	Several agents	Yes	Yes	Yes	No (Yes in non-DKD CKD)	[98-99, 269]

DKD: diabetic kidney disease, CKD: chronic kidney disease, HMGCoA: 3-hydroxy-3-methylglutaryl coenzyme A, RAAS: renin angiotensin aldosterone system, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker; CTGF: Connective tissue growth factor, TGF-beta: Transforming growth factor beta, EGFR: Epidermal growth factor receptor, CXCR4: Chemokine Receptor type 4, CCL2: Chemokine Ligand 2

Inflammation and microbiota

Inflammation is a critical component of diabetes-induced target organ injury and of CRC initiation and progression [112, 113]. In preclinical models of T2DM, inflammation contributed to carcinogenesis and tumor growth, which were prevented by TNF-neutralizing monoclonal antibodies [57].

Multiple signaling pathways are involved in the inflammatory response, including MAPK, NF-κB, janus kinase/signal transducer and activator of transcription (JAK/STAT) and hypoxia-inducible factor-1α [68, 114-117]. Persistent NF-κB/IL-6/STAT3 activation promotes colitis associated CRC [118]. The non-canonical NF-κB pathway has also been implicated in diabetes complications and cancer [119-121]. The upstream kinase of this pathway, NIK, contributes to β cell failure in diet-induced obesity [122], promotes kidney injury [123] and underlies the sensitivity of $Nlrp12^{-/-}$ mice to gut inflammation and tumorigenesis [124]. These intracellular pathways amplify inflammatory responses and promote angiogenesis, cancer growth and invasiveness of malignant cells [125, 126], as well as progression of diabetes target organ injury such as DKD [117].

The interaction between colon epithelial cells and the microbiota may confer susceptibility to both colon cancer and obesity. The inflammasome regulates the microbiota and the inflammatory response of epithelial cells to the microbiota. Deficiency in inflammasome components (e.g. Nlrp6) is associated with an abnormal microbiota, exacerbated gut inflammatory responses [127] and colon tumorigenesis [128] dependent on microbiota-induced activation of epithelial IL-6 signaling [17]. Microbiota-dependent inflammatory responses may contribute to non-Mendelian familial aggregation of colon cancer since in preclinical models the risk of cancer was transmissible between co-housed individuals with the microbiota. The gut microbiota also impacts host metabolism, facilitating obesity, insulin resistance and T2DM [129]. Thus, inflammasome deficiency-related changes in gut microbiota are associated with insulin resistance and obesity [130]. In this regard, T2DM is one of three models of microbiome-associated human conditions to be studied by the Integrative Human Microbiome Project (iHMP, http://hmp2.org) [131].

Table 5: Key points

1. An epidemiological association has been reported between diabetes mellitus, especially type 2 diabetes mellitus , and colorectal cancer
    a. However, the association has evolved over time, there are differences between countries over the impact of sex, and colorectal cancer remains uncommon in many countries with a high prevalence of diabetes, suggesting the existence of poorly understood modifiers.
2. The mechanistic basis for this association are poorly understood
    a. There are common risk factor for colorectal cancer, diabetes and diabetic complications
    b. There are controversial observational data on the association of antidiabetic drugs with colorectal cancer. The most convincing evidence is on a protective effect of metformin
    c. Preclinical data suggest that the diabetic environment may promote both colorectal cancer and diabetic complications.
3. There is evidence derived from interventional preclinical studies, GWAS studies, and some interventional clinical data that suggest that CRC and well-characterized complications of diabetes, such as diabetic kidney disease, may share pathogenic pathways, including inflammatory mediators, an abnormal microbiota and altered iron metabolism, some of them converging at Wnt/β-catenin signaling and MIR-21.
4. The finding of common pathogenic pathways for colorectal cancer and diabetic target organ complications (e.g. diabetic kidney disease) lend biological plausibility to the epidemiological observation

However, to date no clinical practice consequence has derived from this knowledge

Human T2DM and CRC share some microbiota features, such a decrease in the abundance of butyrate-producing bacteria [18, 132]. Butyrate is a breakdown product of dietary fiber that has anti-tumorigenic properties and is associated with decreased incidence of CRC [18]. In mice, the microbiota potential for butyrate production negatively correlated with tumor count [133]. Butyrate also has nephroprotective properties in DKD [134].

Iron metabolism. Altered iron metabolism facilitates rapid proliferation in cancer cells [135]. Indeed, constitutive Wnt/β-catenin signaling in colon cancer cells is iron-dependent [136] and iron chelation limits cell proliferation and has anti-inflammatory effects through NF-κB blockade [137]. Iron overload causes DM and is present in target organs of diabetes, such as the kidneys, while iron depletion upregulates glucose uptake and insulin signaling in liver and decreases kidney inflammation in experimental diabetes [138-140]. Indeed, the Trial to Assess Chelation Therapy (TACT) disclosed a benefit of ethylenediaminetetraacetic acid (EDTA), a chelator that also binds iron, on cardiovascular outcomes, especially in DM patients [141]. Thus, excess cellular iron may facilitate CRC growth, DM and DM complications. Heme iron may be the common denominator in the association of red meat intake with both DM and CRC [142, 143].

Epigenetic changes

CRC and DM also share some epigenetic changes. Thus, both CRC and DM were associated with a positive septin 9 (SEPT9) DNA-methylation assay (Epi-proColon) result [144]. In this regard, SEPT9 is differentially methylated in human T2DM islet cells and was shown to perturb insulin and glucagon secretion [145].

miRNAs are small non-coding RNA molecules that regulate gene expression. Pathogenic miRNAs may be shared by CRC and DKD [146-148]. In murine DKD, renal miR-21 expression was increased and miR-21 knockdown ameliorated renal damage [149]. The pathogenic potential of miR-21 is supported by some, but not all additional reports [150, 151]. miR-21 is also part of a six-miRNA-based classifier that reliably predicts CRC recurrence [148, 152]. Functional studies support a role for miR-21 in colon cancer proliferation and invasion [153, 154] and targeting miR-21 enhanced the sensitivity of human colon cancer cells to chemoradiotherapy and reduced angiogenesis [154, 155]. Metformin synergy with 5-fluorouracil and oxaliplatin to induce death of chemoresistant colon cancer cells was also associated with a reduction in miR-21 [156].

Table 6: Standing questions on the relationship between DM and colorectal cancer

Standing question	Relevance	What is required to address it
Is there an association between T2DM and colorectal cancer across all countries and cultures?	Provides insights into etiologic and pathophysiologic factors, may prevent a colorectal cancer epidemic in the developing world	Head-to-head comparison between developed and developing country cohorts
Is there an association between development of cancer and development of other complications of DM?	Provides the epidemiological basis to search for common mediators of disease	Epidemiological studies, ideally prospective
What molecular mediators explain the association between DM and cancer? Are they shared by other complications of DM?	Identification of potential diagnostic signatures and therapeutic targets	Interventional preclinical models that address function of key molecules. These may have been identified by non-biased systems biology approaches and hypothesis-driven studies designed from the analysis of available literature
Has DM-associated colorectal cancer a specific molecular signature?	This may identify diagnostic signatures and therapeutic targets specific for DM-associated colorectal cancer	Systems biology comparison between DM and non-DM associated colorectal cancer with DM and non-DM healthy colon as control
Can DM patients at high risk for cancer development be identified by diagnostic tests?	Early diagnosis of risk or cancer	Prospective systems biology approach to relevant biological samples (feces, urine, blood or others)
Can DM patients at high risk or early colorectal cancer be treated by specific, DM-tailored approaches? Do these approaches also prevent/treat other diabetic complications?	New preventive/therapeutic approaches that address both cancer and non-cancer DM complications	Early identification of patients at high risk or with early disease Unraveling of common pathogenic pathways
Are there common microbiota signatures for colorectal cancer and other DM complications?	New preventive/therapeutic approaches that address both cancer and non-cancer DM complications	Metagenomic studies
What is the optimal therapeutic approach for colorectal cancer in diabetic individuals and the optimal therapeutic approaches for DM in colorectal cancer patients?	Therapy individualization and improved outcomes	Hypothesis-generating observational studies followed by randomized clinical trials

ADDITIONAL INFORMATION FROM SYSTEMS BIOLOGY APPROACHES

Genome-wide association studies (GWAS) have identified susceptibility genes for DM or CRC that provide insights into potentially shared pathogenic pathways, such as *TCF7L2, KCNQ1, HMGA2, RHPN2* and *GREM1*.

TCF7L2 harbors common genetic variants with the strongest effect on T2DM risk [157-159] and on DM complications such as DKD [160] and is also susceptibility locus for CRC loci in East Asians [161]. TCF7L2 is a transcription factor and β-catenin transcriptional partner in the Wnt-signaling pathway. DNA-bound TCFs repress gene transcription in the absence of β-catenin, but are required for β-catenin transcriptional activity [162]. TCF7L2 also promotes miR-21 expression [163]. Another CRC-associated Single Nucleotide Polymorphism (SNP), rs6983267, is located at a TCF7L2 binding site and the risk allele results in stronger TCF7L2 binding, facilitating Wnt signaling [164]. A common *GREM1* SNP, rs16969681, associated with CRC susceptibility facilitates TCF7L2 binding to DNA leading to stronger gene expression [165]. A germline duplication upstream of *GREM1* causes hereditary mixed polyposis syndrome and Mendelian-dominant predisposition to CRC through ectopic *GREM1* overexpression in the intestinal epithelium [166, 167]. *GREM1* was initially identified as one of the most upregulated genes in cultured mesangial cells exposed to high glucose [168] and *GREM1* gene variants also associate with DKD [169]. Gremlin, the protein codified by *GREM1*, has been proposed as a key mediator of DKD [170-173]Gremlin promotes the motility of CRC cells [174] and the epithelial to mesenchymal transition in kidney tubular cells, also associated with increased motility [175, 176]. The precise role of TCF7L2 in CRC should be further defined. Thus, *TCF7L2* mutations identified in cancer samples abolish its ability to function as a transcriptional regulator and result in increased CRC cell growth [177]. Given the multitude of target genes, this is not surprising.

KCNQ1 was associated with T2DM [178]. This locus encodes both KCNQ1 and the long noncoding RNAs (lncRNAs) *KCNQ1OT1*, which is a β-catenin target dysregulated in CRC [179]. In human CRC, low *KCNQ1* expression was associated with poor survival and mutation of the murine homologue Kcnq1 increased the risk for intestinal tumors [180].

HMGA2 is a further gene associated to risk of T2DM and DKD in GWAS [158, 181]. *HMGA2* expression is increased in and promotes the malignant behavior of CRC [182, 183]. Conversely, CRC GWAS identified *RHPN2* as a susceptibility loci and *RHPN2* expression is upregulated in experimental DKD [184, 185].

Pathway-based enrichment analysis of 23

independent gene expression profiling studies on prognosis of CRC observed overrepresentation of the oxidative phosphorylation chain, the extracellular matrix receptor interaction category, and a general category related to cell proliferation and apoptosis [186]. These categories are functionally related with cancer progression. Eight of the genes were also present in a previous meta-analysis of ten expression profiling studies of differentially expressed genes in CRC with good versus bad prognosis, including *IQGAP1, YWHAH* and *TP53*. [186]. IQGAP1 is part of the podocyte filter for proteins and regulates the occurrence of proteinuria, the hallmark of DKD [187] and *YWHAH* expression was increased in human DKD transcriptomics studies [188, 189]. Furthermore, human DKD transcriptomics revealed that 25% of apoptosis-related genes were differentially regulated in kidney tissue [190-192]. Some of the specific factors identified by human DKD transcriptomics and functionally characterized to contribute to kidney injury, also promote CRC growth, such as the MIF/CD74 system which is under study as a therapeutic target in colon cancer [193, 194]. Furthermore, elements of the JAK/STAT, VEGFR signaling and inflammation-related pathways were also overrepresented in human DKD [184, 189]. JAK/STAT, VEGF and inflammation are therapeutic targets in cancer.

As part of the Human Proteome Project, the Biology/Disease-driven Human Proteome Project (B/D-HPP) consortium leads specific projects on diabetes (HDPP) and cancer that may shed some additional light on the relationship between both diseases [195]. Protein candidate markers responding to CRC existence (diagnosis), stratification (different response related to stage) or prognosis (survival/metastasis) have been identified [196-199]. Most studies compared normal (healthy) tissue with tumor. The top four regulated proteins in a systematic review of CRC were 60-kDa heat shock protein (HSP60) and Nucleoside diphosphate kinase A (nm23-H1), up-regulated, and Selenium-binding protein 1 (SELENBP1) and Carbonic anhydrase I (CAI), down-regulated [200]. Interestingly, expression of the HSPD1 gene encoding HSP60 was upregulated and SELENBP1 downregulated in human DKD, according to the Nephromine database, further suggesting potential common pathogenic pathways between DKD and colon cancer (http://www.nephromine.org/).

Bioinformatics approaches may be used to integrate the growing systems biology databases. One such approach, the Drug-specific Signaling Pathway Network (DSPathNet) was used to tentatively identify seven genes (CDKN1A, ESR1, MAX, MYC, PPARGC1A, SP1, and STK11) and one novel MYC-centered pathway that might play a role in metformin antidiabetic and anticancer effects [201]. Interestingly, PPARGC1A protects from kidney injury and the expression is downregulated by inflammation [202].

IMPLICATIONS FOR THERAPY

Given the high and increasing incidence and prevalence of DM and CRC, it is likely that, independently from any common pathogenic pathways or associations, many DM patients will develop CRC. This brings the question whether physicians need to modify the approach to therapy of DM or CRC in diabetic patients with both conditions. In this regard, a diagnosis of cancer is frequently associated to a subsequent decrease in adherence to antidiabetic medication [203].

Choice of antidiabetic agent in the patient with CRC

The ADA Standards of Medical Care in Diabetes indicates that patients with DM should be encouraged to undergo recommended age- and sex-appropriate cancer screenings and to reduce their modifiable cancer risk factors (obesity, smoking, physical inactivity) [9]. In the presence of cancer, higher HbA1c goals should be considered: <8% in the absence of metastases and <8.5% for patients with metastatic cancer. If indeed hyperglycemia underlies the higher incidence of colon cancer in DM, these higher thresholds may theoretically impair cancer-related outcomes.

ADA 2016 does not provide recommendations on the choice of antidiabetic treatment in patients with cancer or CRC [204]. However, the initial antidiabetic agent recommended for standard T2DM patients, metformin, has been associated with decreased incidence or better outcomes in cancer patients [205-207]. Thus, even if prospective clinical studies confirmed the superiority of metformin on cancer incidence or outcome, this would not change the current standard therapeutic approach for T2DM in the cancer patient. The debate about the association of specific antidiabetic drugs and cancer risk has been marred by the lack of properly designed studies.

Although observational studies suggest that the choice of treatment for DM may modify cancer risk [208], no prospective studies have been specifically designed to address this issue. Thus, no firm conclusions can be reached at this point. The crux of the debate has been whether insulin or analogs are associated to an increased risk of CRC (and cancer in general) [209] and whether metformin is associated with a decreased risk of CRC [210]. This may represent two sides of the same coin: if one drug does modify the risk of CRC, by comparison the other may appear to modify the risk in the opposite direction. Confounders may exist. Thus, insulin is generally prescribed and metformin remains formally contraindicated in advanced chronic kidney disease (CKD), a late event in the course of T2DM, despite recent clinical recommendations [211]. Renal insufficiency is associated with higher risk for all-cause cancer [212],

although this association has not been demonstrated for CRC [213].

Recent meta-analyses have attempted to unravel the potential relationship between antidiabetic therapy and cancer or colorectal cancer. However, meta-analysis results heavily depend on the quality of the included studies. A recent meta-analysis involving approximately 7.6 million and 137, 540 patients with diabetes from observational studies and randomized controlled trials (RCTs), respectively, suggested that metformin or thiazolidinediones were associated with a lower risk of all cancer incidence, while insulin, sulfonylureas and alpha glucosidase inhibitors were associated with an increased risk of cancer incidence [214]. Another large (491, 384 individuals) meta-analysis addressing specifically the impact of insulin, found it to be associated with a significant 69% increased risk of CRC in T2DM only in case-control but not in cohort studies [215]. The Barcelona nested case-control study of 275, 164 T2DM patients did not find an increased risk of cancer for any insulin or oral antidiabetic agent [216]. Finally, a metaanalysis of 19 publications representing data for 1, 332, 120 individuals, insulin had no effect and insulin glargine was associated with a decreased risk of CRC [217].

Metformin use has been associated with a decreased risk of colon cancer and increased survival [210, 218, 219]. A systematic review of 12 randomized controlled trials (21, 595 patients) and 41 observational studies (1, 029, 389 patients) found that in observational studies the risk of CRC was 17% lower among DM patients treated with metformin than in those not on metformin [210]. In a meta-analysis of 21 observational studies metformin was associated with a reduction in cancer-specific mortality, including a reduction in mortality for colon cancer (4 studies, HR 0.65, 0.56-0.76) [205, 220]. Several mechanisms may account for the antitumor effect of metformin. It reduces circulating insulin, promotes weight loss and activates 5' adenosine monophosphate-activated protein kinase (AMPK), thus inhibiting growth of colon cancer cells [221, 222]. In mice with Apc mutations, metformin suppressed polyp growth [223] and in diabetic mice metformin, alone or in combination with oxaliplatin, reduced the severity of colorectal tumors [224]. Older literature described increased expression of mitochondrial GPDH, the target of metformin, in rapidly growing, undifferentiated tumors [225, 226]. However, there are no data on CRC expression of mitochondrial GPDH. In non-diabetic subjects, oral short-term low-dose metformin suppressed the development of colorectal aberrant crypt foci in a clinical trial [227]. In phase 3 RCT, low-dose (250 mg/day) metformin was safe and reduced the prevalence and number of metachronous adenomas or polyps after polypectomy in non-diabetic patients [228].

Conflicting results are available on thiazolidinediones and cancer. A systematic review and meta-analysis of 840, 787 diabetic patients did not support an association between thiazolidinediones and CRC [229, 230]. In a 6-year population-based cohort study, thiazolidinediones were associated with decreased cancer risk including CRC and the association was dose-dependent [231]. Thiazolidinediones have cytostatic effects and inhibit growth and metastasis of colon cancer cells as they induce differentiation and modulate the E-cadherin/β-catenin system [232-234]. However, some studies point to a mitogenic potential of troglitazone which induced colon tumors in normal C57BL/6J mice and increased colon carcinogenesis in Apc1638 N/+Mlh1$^{+/-}$ double mutant mice [235].

In systematic meta-analyses, sulphonylureas were associated with increased risk of pancreatic and hepatocellular cancer but not of CRC [229, 236, 237]. A cohort of 275, 164 T2DM patients found no evidence for altered cancer risk for repaglinide or α-glucosidase inhibitors compared to insulin-based therapies or other oral glucose-lowering drugs [216]. In other reports, acarbose was associated with reduced the risk of incident CRC in patients with diabetes in a dose-dependent manner [238, 239]. Acarbose may alter the microbiota [240] and decreased the size of gastrointestinal adenomas in Apc knockout mice [241].

Empaglifozin dramatically decreased mortality and slowed DKD progression and sodium-linked glucose transporter-2 (SGLT2) inhibitors may soon become the new standard of therapy [242]. A safety warning was issued by the FDA regarding bladder and breast cancer risk from early clinical trials of dapagliflozin but not for CRC (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM262994.pdf). Adenocarcinomas express SGLT2 and SGLT2 inhibitors blocked glucose uptake and reduced growth of tumor xenografts [243]. Whether this applies to CRC is unknown.

No relationship between GLP-1-based therapies and CRC have been reported [244, 245]. However, exenatide inhibited proliferation and induced apoptosis in cultured murine CT26 colon cancer cells [246, 247].

Choice of chemotherapy for colorectal rectal cancer in patients with diabetes

Studies addressing chemotherapy efficacy or safety in DM are very limited and there is no evidence supporting specific chemotherapy approaches for CRC patients with DM. No differences in the survival benefit or severe adverse effects associated to chemotherapy were observed in 5, 330 elderly CRC patients with (n=950) and without (n=4, 380) DM [248]. By contrast, a cohort study within the INT-0089 randomized adjuvant chemotherapy trial of 3, 759 patients with high-risk stage II/III colon cancer concluded that in DM patients overall mortality and cancer

recurrence were higher than in non-diabetic patients [249]. Treatment-related toxicities were similar between DM and non-DM patients, except for a higher risk of treatment-related diarrhea among DM patients [249]. However, disease-free survival was lower and neurotoxicity more frequent in DM patients treated with capecitabine and oxaliplatin (CAPOX) chemotherapy than in non-diabetics [250]. It is likely that whether DM modifies the risk of severe adverse effects that limit chemotherapy depends on the specific chemotherapeutic regimen.

AGENTS IN THE PIPELINE TARGETING BOTH CRC AND DIABETIC COMPLICATIONS

Some therapeutic targets are undergoing or have undergone RCTs in both diabetes complications (e.g. DKD) and cancer or are in clinical use in one condition and have been successfully used for the other condition in preclinical settings. These include statins, renin angiotensin aldosterone system (RAAS) blockers, endothelin receptor antagonists, VDR activators, mTOR inhibitors, anti-inflammatory molecules and inhibitors of EGF ligands/receptors (Table 4) [251, 252].

Statins are commonly used to treat hyperlipidemia and have been linked with a small reduction in the risk for colon cancer in diabetic patients [253] and improved prognosis of curatively resected CRC [254]. In an obesity-related colon cancer model associated with hyperlipidemia and hyperinsulinemia, pitavastatin prevented carcinogenesis and inhibited colon proliferation and inflammation [255], while simvastatin inhibited the release of inflammatory cytokines by colorectal cell lines [256]. Clinical trials are exploring statins in the treatment of human CRC.

RAAS blockers are the mainstay of therapy for human DKD [252]. Angiotensin-converting enzyme inhibitors and angiotensin-II type 1 receptor blockers suppress chemically-induced colonic preneoplasic lesions in diabetic animals [257-259]. However, their clinical use to prevent colon cancer is not being pursued.

The endothelin receptor antagonist atrasentan is undergoing RCTs for DKD [260, 261], and as add-on to docetaxel and prednisone for stage IV hormone therapy-resistant prostate cancer bone metastases (NCT00134056) [262-264]. However, no trial is exploring CRC.

Paricalcitol is a VDR activator that may have antiproteinuric effects on DKD as suggested by RCTs [265] and may also slow cancer cell growth [266]. Phase I trials have tested combinations of paricalcitol and chemotherapeutic agents (NCT00217477). Additionally, vitamin D has been explored for colon cancer prevention. However, a combination of calcitriol, aspirin, and calcium carbonate or vitamin D/calcium did not prevent recurrence of colorectal adenomas over a 3- to 5-year period [267, 268].

mTOR inhibitors are used as anticancer agents and also improve experimental DKD [99, 269]. The mTOR inhibitors *RAD001 (*NCT01058655) and *everolimus and the* dual PI3K/mTOR inhibitor *PF-05212384 (*NCT01937715, NCT01154335*) are undergoing clinical trials for m*etastatic CRC.

Several agents targeting cytokines and chemokines have been tested both in T2DM and cancer [251, 252, 270-273]. Plerixafor is a CXCR4 antagonist undergoing trials for advanced CRC (NCT02179970). Although not specifically tested in DM, a selective CXCR4 antagonist AMD3465 decreased mineralocorticoid-dependent renal fibrosis in mice [274] and targeting CXCR4 prevented glomerular injury associated to high podocyte CXCR4 expression in mice [275].

JAK2 targeting prevented high-glucose-induced fibrogenic responses in renal cells and prevented kidney and vascular injury in experimental diabetes [276-279]. An ongoing phase II RCT is testing the JAK1 and JAK2 inhibitor baricitinib as add-on to RAS blockade in patients with DKD (NCT01683409) while another will explore the JAK2/FLT3 inhibitor pacritinib in patients with refractory CRC and KRAS mutations (NCT02277093).

UNANSWERED QUESTIONS AND THE WAY FORWARD

Table 5 summarizes the key points of the review. The association between DM and CRC is recognized by scientific consensus [4]. However, a number of issues require more detailed studies (Table 6).

An overview of T2DM and CRC country-based prevalence/incidence suggests that environmental, development-associated or other factors may interact with the T2DM milieu to increase the risk of CRC. Identification of these putative factors and whether DM associates with increased CRC risk in different cultures and countries may provide further insights into mechanisms underlying the relationship between DM and cancer.

The case for a causal association should be strengthened by the characterization of the DM-initiated molecular pathways involved. This information may also lead to the development of specific preventive or therapeutic approaches. Studies should address the relationship between DM-associated CRC and the development of other DM-associated complications, i.e., whether there is a patient profile prone to develop any DM-related complications. If this were the case, tools should be developed for the early identification of such patients. Urine proteomics holds promise in this regard, as it allows identification of DKD at earlier stages than currently available methods and predicts progression [280, 281] and may also be useful for the diagnosis of cancer outside the urogenital system [282]. Early identification of the subpopulation of DM patients at highest risk for

developing cancer or classical complications may allow enrollment in trials assessing the efficacy of drugs targeting shared molecular mechanisms for prevention and/or therapy. Additional systems biology approaches may also contribute to define molecular pathways leading to DM-associated cancer or target organ damage. The most promising approaches should undergo clinical trial testing, ideally in high-risk populations or in early disease stages identified by the study of specific molecular signatures.

Research is needed to define the optimal therapeutic approach for the patient with T2DM and CRC. Studies of the impact of different antidiabetic agents on cancer incidence are marred by the fact that both sides of the comparison may theoretically modulate cancer incidence. Additionally, there are potential biases related to the indication of the specific agent. These research efforts have the potential to decrease the incidence of DM-associated complications and to improve outcomes. The DiabetesCancerConnect Consortium funded by the Spanish Government is attempting to answer some of these questions.

Abbreviators

DM-Diabetes mellitus
CRC-Colorectal cancer
ADA- American Diabetes Association
T1DM- Type 1 DM
T2DM- Type 2 DM
MODY─ Maturity-onset diabetes of the young
GLP-1- Glucagon like peptide-1
GPDH- Glycerophosphate dehydrogenase
GPD2- Glycerol-3-phosphate dehydrogenase 2
GSK-3β- Glycogen synthase kinase 3-beta
EGFR- Epidermal growth factor receptor
HR- Hazard Ratio
SIR- Standardized incidence ratios
RR- Relative risk
CI- Confidence interval
BMI- Body mass index
OR- Odds Ratio
HbA1c- Glycated hemoglobin
AGEs- Advanced Glycation End-products
RELMβ- Resistin-like molecule β
IGF-1- Insulin-like growth factor
MAPK- Mitogen activated protein kinase
PI3K- Phosphatidyl-inositol-3-kinase
PAK-1- Activated protein kinase-1
mRNA- Messenger RNA
ChREBP- Carbohydrate response element-binding protein
NF-κB- Factor-kappa B
Nrf2- Nuclear factor erythroid 2–related factor 2
ATP- Adenosine triphosphate
Glut-1- Glucotransporter-1
TGF- Transforming growth factor
VDR- Vitamin D receptor
PTHrP- Parathyroid hormone-related protein-
Grem1- Gremlin
S1P- Sphingosine-1-phosphate
iHMP- Integrative Human Microbiome Project
TACT- Trial to Assess Chelation Therapy
EDTA- Ethylenediaminetetraacetic acid
miRNAs- MicroRNAs
GWAS- Genome-wide association studies
lncRNA- Long non-coding RNA
SNP- Single Nucleotide Polymorphism
eQTL- Expression quantitative trait loci
KEGG- Kyoto Encyclopedia of Genes and Genomes
VEGFR- Vascular endothelial growth factor receptor
B/D-HPP- Biology/Disease-driven Human Proteome Project
HDPP- Human Diabetes Proteome Project
SELENBP1- Selenium-binding protein 1
CAI- Carbonic anhydrase I
DSPathNet- Drug-specific Signaling Pathway Network
RCTs- Randomized controlled trials
CKD- Chronic kidney disease
ROS- Reactive Oxygen species
PPARγ- peroxisome proliferator–activated receptor γ
DNA- Deoxyribonucleic acid
RNA- Ribonucleic acid
FDA- Food and Drug Administration
CAPOX- Capecitabine and oxaliplatin
RAAS- Renin angiotensin aldosterone system
mAb- Monoclonal antibody
HG- Hyperglycemia
DKD- Diabetic kidney disease
ARB- Angiotensin receptor blocker
HMGCoA- 3-hydroxy-3-methylglutaryl coenzyme A
ACE- Angiotensin converting enzyme-
iNOS- Inducible nitric oxide synthase
NF-κB- Nuclear factor kappa-light-chain-enhancer of activated B cells
ERK- Extracellular Signal-regulated Kinase
CTGF- Connective tissue growth factor
TGF-beta- Transforming growth factor beta
CXCR4- Chemokine Receptor type 4
CCL2- Chemokine Ligand 2

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CONFLICT OF INTERESTS

The authors have no competing financial interests.

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PLOS ONE

RESEARCH ARTICLE

Colon cancer modulation by a diabetic environment: A single institutional experience

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Abstract

Background

Multiple observational studies suggest an increased risk of colon cancer in patients with diabetes *mellitus* (DM). This can theoretically be the result of an influence of the diabetic environment on carcinogenesis or the tumor biologic behavior.

Aim

To gain insight into the influence of a diabetic environment on colon cancer characteristics and outcomes.

Material and methods

Retrospective analysis of clinical records in an academic tertiary care hospital with detailed analysis of 81 diabetic patients diagnosed of colon cancer matched with 79 non-diabetic colon cancer patients. The impact of streptozotocin-induced diabetes on the growth of colon cancer xenografts was studied in mice.

Results

The incidence of DM in 1,137 patients with colorectal cancer was 16%. The diabetic colon cancer cases and non-diabetic colon cancer controls were well matched for demographic and clinical variables. The ECOG Scale Performance Status was higher (worse) in diabetics (ECOG $\geq$1, 29.1% of controls vs 46.9% of diabetics, p = 0.02), but no significant differences were observed in tumor grade, adjuvant therapy, tumor site, lymphovascular invasion,

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Abbreviations: ADA, American Diabetic Association; ECOG, Eastern Cooperative Oncology Group; BMI, Body Max Index; pT, Invasion depth or thickness of the tumor; AJCC, American Joint Committee on Cancer; STZ, streptozotocin; CEA, carcinoembryonic antigen; FBS, Fetal Bovine Serum.

stage, recurrence, death or cancer-related death. Moreover, no differences in tumor variables were observed between patients treated or not with metformin. In the xenograft model, tumor growth and histopathological characteristics did not differ between diabetic and nondiabetic animals.

Conclusion

Our findings point towards a mild or negligible effect of the diabetes environment on colon cancer behavior, once cancer has already developed.

Background

Diabetes *mellitus* and cancer are among the most frequent causes of death worldwide [1–3]. Epidemiologic evidence suggests an association between diabetes *mellitus* and an increased risk of many forms of cancer [4–6]. Colon cancer is the third -in men- and the second -in women- most common cancer in the global population [2]. The results of multiple observational studies, but not all, have suggested that diabetes may significantly increase the risk of colon cancer [7–11]. However, data from specific studies have been disputed as based on heterogeneous groups, with unreliable ascertainment of diabetes, and using sources with poorly rigorous data such as Surveillance, Epidemiology, and End Results, which may have led to inconclusive or misleading results [9, 12–14]. Accordingly, the magnitude of the association may have been potentially influenced by hints and bias [15, 16]. It also remains uncertain whether the putative link between diabetes and colon cancer is direct, related to hyperglycemia; whether diabetes is an indicator of latent biologic factors that modify cancer risk such as insulin resistance and hyperinsulinemia; or whether the diabetes-cancer association is indirect and reflects the influence of common risk factors such as age, lifestyle behaviors, overweight, poor dietary habits or therapeutic agents [17]. Moreover, there is a limited knowledge related to the influence of a diabetic environment on the carcinogenesis process, the biologic behavior of the tumor, the response to different treatments and patient outcomes [5, 18]. *In vitro* and *in vivo* studies have supported a direct role of high glucose concentrations in tumor development and characteristics [19]. In this respect, animal models have addressed the influence of diabetes/insulin resistance on carcinogenesis (e.g. carcinogenic molecules used in Leptin receptor–deficient db/db mice) [20], or of diabetes/hyperglycemia on tumor xenograft progression (e.g. glucagon-induced hyperglycemia [21] or streptozotocin-induced diabetes in mice [22, 23]), although colon cancer had not been previously studied in depth.

We have now aimed at gaining insight into the influence of a diabetic environment on colon cancer characteristics and progression through a combination of clinical studies and a preclinical evaluation of the influence of the hyperglycemia on tumor xenograft progression.

Material and methods

Clinical study

The study is a retrospective patient record analysis carried out at Fundacion Jimenez Diaz Hospital, for patients from January 2009 to December 2013. Clinical data and biopsies were obtained after informed consent from patients underwent surgery for purposes not related to this study. Candidate patients were identified using the specific institutional software named Alcor (Sigesa, 2014). This program works with an encoded system, which matched the specific

requested terms. The first research setting included the variables colorectal cancer and diabetes, and the second diabetes, cancer and colorectal cancer.

Study sample. From the total number of patients found with a diagnosis of colorectal cancer and also diabetes at the institution, only 81 diabetic patients met the inclusion criteria established for this study:

Patients with resection of primary colon cancer, histological type colon adenocarcinoma, colon location (rectal cancer patients were excluded), time from surgery up to 6 months, no neoadjuvant treatment, no other concurrent neoplasia or immunosuppressive treatment, and diabetes diagnosed as a documented registry of diabetes, or historical anti-diabetic medication intake or meeting the American Diabetic Association (ADA) criteria for diabetes at time of reviewing the data. The ADA criteria used to determine if patients had diabetes were: Hemoglobin A1c values $\geq$ 6.5%, or fasting blood glucose levels $\geq$ 125 mg/dL, with high fasting values recorded 2 or more times or random blood glucose levels $\geq$ 200 mg/dL, with high random values recorded 2 or more times (S1 Fig).

In parallel, this observational study also included 79 non-diabetic patients with primary diagnosis of colon cancer, who underwent resection during the same period, using equal inclusion criteria except the presence of diabetes, aiming to obtain a well-balanced series.

Baseline variables recorded for all patients were age, gender, metformin intake, clinical debut (acute or subacute symptoms), Eastern Cooperative Oncology Group (ECOG) Scale Performance Status, Body Mass Index (BMI), adjuvant therapy, overall survival, disease free survival, and cause of death if deceased. Other variables recorded from peripheral blood at diagnosis were glucose, triglycerides, cholesterol, white blood cell levels, and carcinoembryonic antigen (CEA) level.

Tumor characteristics were analyzed based on the following criteria:

- Information regarding depth of invasion or thickness of the tumor (pT) was collected from the pathology reports after surgery, as defined by the American Joint Committee on Cancer Criteria for colon and rectal cancer staging (https://cancerstaging.org/About/Pages/8th-Edition.aspx).
- Tumors were graded as low grade (G1-G2), and high grade (G3) following the 2010 WHO classification (http://www.pathologyoutlines.com/topic/colontumorwhoclassification.html).
- Tumor location was categorized as proximal or right (cecum, hepatic flexure, ascending and transverse colon), and distal or left (splenic flexure and descending colon) [24].
- Information regarding lymphovascular invasion was collected from the pathology report, determined on hematoxylin and eosin staining. Vein invasion was identified as present if tumor cells were observed in an endothelial-lined channel with a smooth muscle wall. Lymphatic vessel invasion was identified as present if tumor cells were observed in an endothelial-lined channel devoid of smooth muscle.
- TNM staging: Data on Classification of Malignant Tumours (TNM) staging was collected from the clinical registries in the computerized system and was assessed based on pathology reports available after surgical resection and results from imaging studies. The information was coded according to the TNM staging described in the American Joint Committee on Cancer (AJCC) eighth edition (https://cancerstaging.org/Pages/default.aspx). For analytical purposes, T stages were classified as low T stage (T1-T2) or high T stage (T3-T4), N stages were considered as N0 (absence of node involvement) and N+ (lymph node metastases) and final stage as low stage (0, I, any II) or high stage (any III, IV).

The study was approved by the Institutional Scientific and Ethical Committee at IIS-Fundacion Jimenez Diaz (Madrid, Spain) (CEIC-FJD, approval code 08/13; on October 1st, 2013) in

accordance with the ethical principles stated in the Declaration of Helsinki. Informed consent is included in the clinical history of each participant and recorded by the standard requirements of data protection rules established by the SPANISH DATA PROTECTION AGENCY (LOPD 15/1999).

Tumor xenograft model in streptozotocin-induced diabetic mice

Fifteen male athymic mice NU-Foxn1nu (8-week-old) were purchased from Charles River Laboratories and housed in the specific pathogen-free room of the Animal Model Core Facility of Research Health Institute–Fundacion Jimenez Diaz (ES28079000089). Mice were maintained in individually ventilated cages (IVCs) with a 12:12 h light-dark cycle. 2014 Teklad global 14% protein irradiated diet and autoclaved water was provided *ad libitum*.

All animal procedures and experimental protocols were approved by the Ethical Animal Research Committee at IIS-Fundacion Jimenez Diaz (Madrid, Spain) and were also conducted in accordance with institutional standards (Reference n˚: PROEXP 024–15), which fulfilled the requirements established by the Spanish government and the European Community (Real Decreto R.D. 53/2003).

Diabetes induction. Diabetes was chemically induced by a single intraperitoneal injection of 200 mg/Kg body weight streptozotocin (STZ, Sigma-Aldrich) in a total volume of 200 μl in 50 mM citrate buffer (pH = 4.5) in 10 animals. The control group (5 mice) received 200 μl citrate buffer. Ten days after the STZ administration, 60% of STZ injected animals presented blood glucose above 200 mg/dl and were considered as the streptozotocin-induced diabetic (STZ-D) group. Blood glucose levels were monitored periodically during the experiment, but animals did not receive insulin or other antidiabetic drug.

Xenograft implantation. The colorectal cancer HT29 cell line, recently classified as "metabolic subtype" [25, 26], was used to generate a xenograft twenty days after streptozotocin or vehicle administration. Cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS) at 37˚C in a 5% CO_2 atmosphere. Medium was supplemented with penicillin G (100 U/ml), and streptomycin (0.1 mg/ml). A volume of 200 μl containing $2x10^6$ cells [1:1 mixture of PBS: Matrigel (BD Biosciences)] was injected subcutaneously into both flanks of the animal. The size of the tumor was measured three times a week using a Vernier caliper along two perpendicular axes and volume was calculated with the formula: volume = (length × $width^2$)/2 (Fig 1).

Pathological assessment. Fifty-five days after tumor induction, mice were sacrificed by using carbon dioxide (CO2) euthanasia and tumors were removed and fixed in 10% neutral buffered formalin solution for 24 hours and embedded in paraffin wax. All samples were processed following the same procedure. Tissues were cut into 4-μm-thick sections, and stained with hematoxylin-eosin stain for morphological examination by light microscopy.

CD31-vascular structures in tumor were also evaluated by immunohistochemistry. Consecutive 4-μm tissue sections were obtained from formalin-fixed paraffin-embedded samples. Antigen retrieval was performed in PT-Link (Dako) for 20 min at 95˚C in high pH buffered solution (Dako). Endogenous peroxidase was blocked by immersing the sections in 0.03% hydrogen peroxide for 5 min. Slides were washed for 5 min with Tris buffered saline solution containing Tween 20 at pH 7.6 and incubated with a primary antibody for CD31 (dilution 1:25, Clone JC70A, Abcam) for 20 min at room temperature, followed by incubation with the appropriate anti-Ig horseradish peroxidase-conjugated polymer (EnVision, Dako). Sections were then visualized with 3, 3'-diaminobenzidine as a chromogen for 5 min and counterstained with hematoxylin. All stainings were performed in an Autostainer platform (Dako). CD31-vascular structures were counted in ten ×200 magnification microscopic fields from the area of highest vascular density in the tumor.

Statistical analysis

Data were analyzed by SPSS v.20.0 software. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequencies and percentages. Demographic, clinical characteristics, and tumor pathologic variables were compared between patients with and without diabetes by chi-square or Fisher´s exact test when appropriate, in case of categorical variables and by t-test in case of continuous variables. A p value lower than 0.05 was considered statistically significant, in all analyses.

Results

Epidemiological data

Among 1,137 patients diagnosed of incident colorectal cancer from January 2009 to December 2013 found, diabetes was present in 185 (16%); the incidence of any cancer in among 13,873 diabetic patients was 14%, being 1.3% of these cases colorectal cancer (Fig 2).

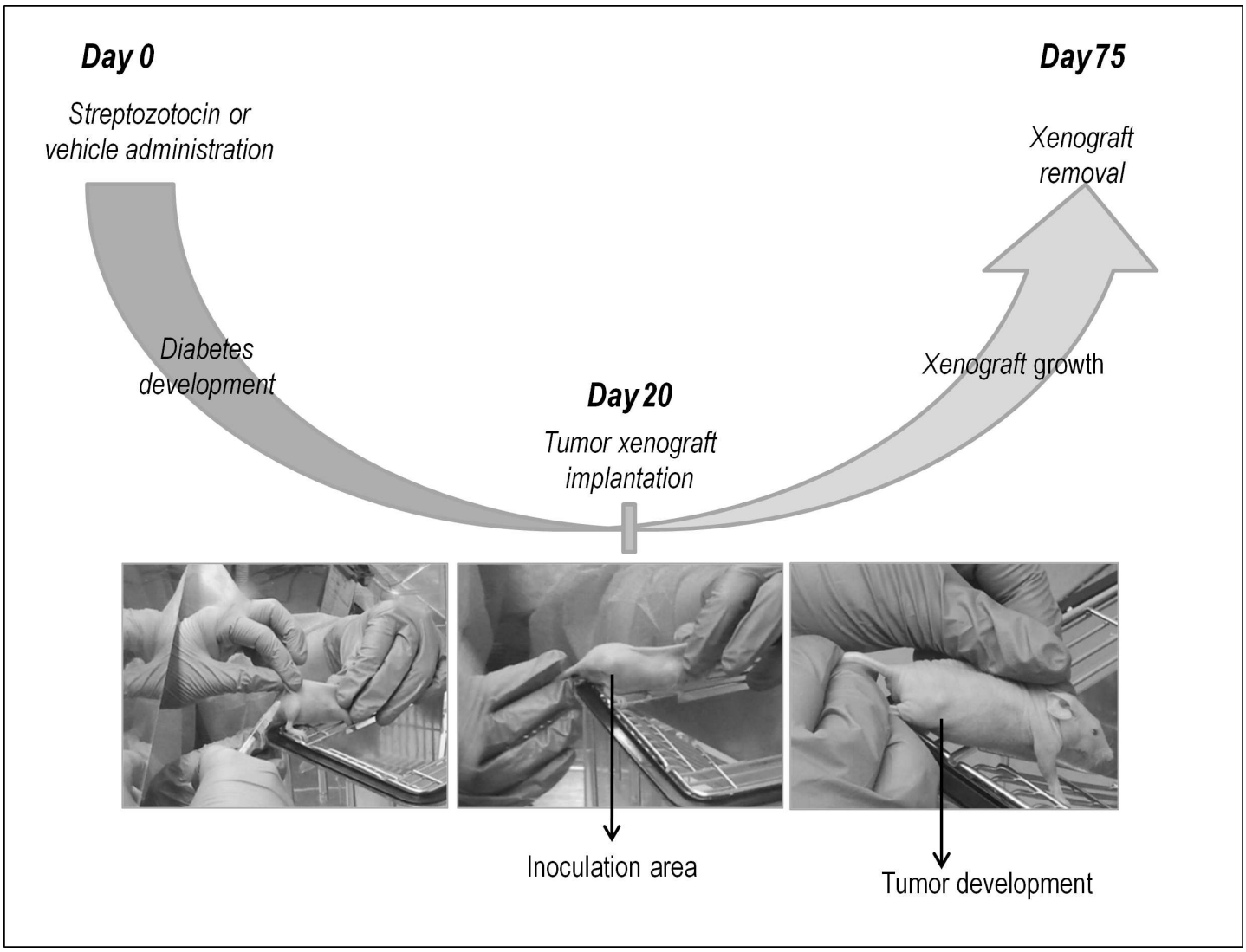

Fig 1. Schematic representation of the cancer xenograft model in mice.

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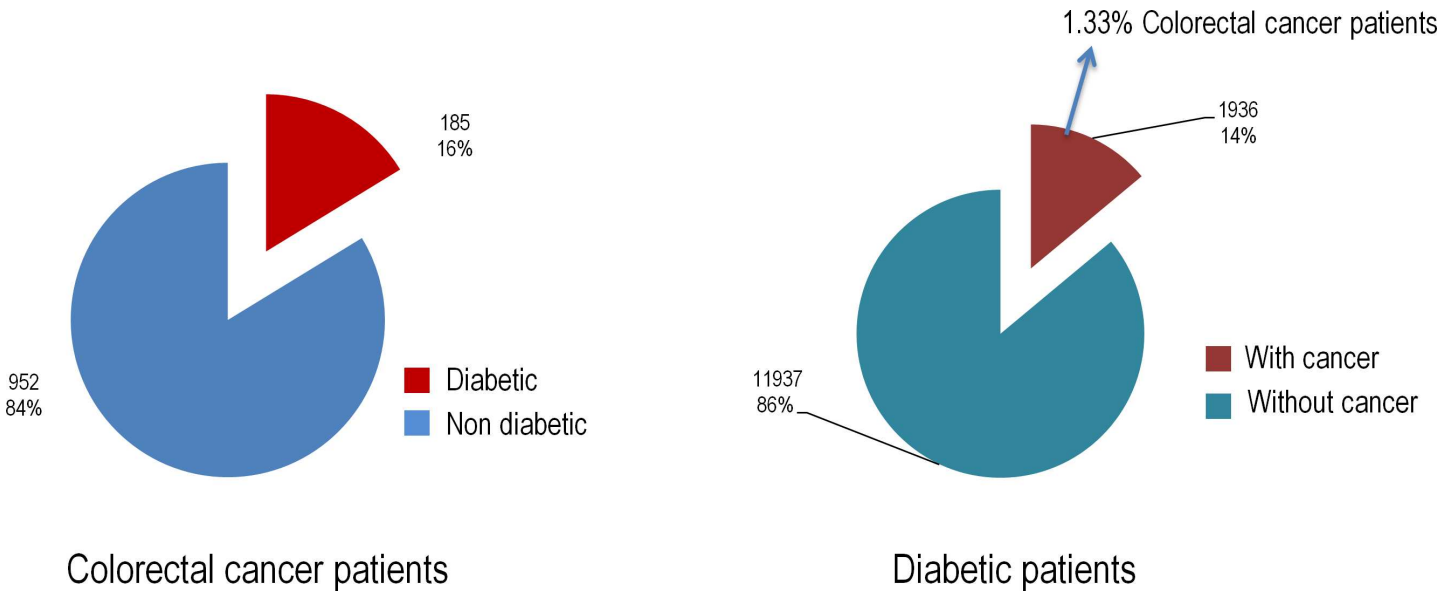

Fig 2. Overview of the stratified data, provided by the medical records of patients at Fundacion Jimenez Diaz.

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Colon cancer characteristics in diabetic versus non-diabetic patients

Descriptive and clinical results and frequencies of variables of diabetic and non-diabetic colon cancer patients that met inclusion criteria are presented in Table 1. Demographic and clinical characteristics in both arms demonstrated the homogeneity of the group. The mean age was 72.9 (range, 45–95 years) for non-diabetic patients and 76.7 (range, 46–91) for diabetic patients.

Results were homogeneous with respect to gender, clinical debut and CEA levels, while serum glucose, triglycerides and circulating neutrophils were significantly higher and serum cholesterol significantly lower in diabetics and there was a non-significant trend towards higher BMI in diabetics.

ECOG Scale Performance Status was 0 in 99 patients (61.9%), and ≥1 in the rest of patients (38.1%); with the diabetic group having higher prevalence of high scores (ECOG ≥1 29.1% in non-diabetics *vs* 46.9% in diabetics, p = 0.02).

Regarding tumor variables, no significant differences were found between diabetics and non diabetics in the prevalence of high T stage, node involvement, grade of differentiation, presence of lymphovascular invasion, tumor location, recurrence rate, death events or cancer-related deaths.

From the total of diabetic patients, 35 were treated with metformin. No significant differences were observed in tumor-related features between diabetic patients on metformin or not on metformin (Table 2).

A diabetic environment does not modify tumor xenograft growth in STZ-D mice

Since clinical data did not support a relationship between diabetes and specific tumor features, the influence of diabetes on the growth and histological features of human colon cancer xenografts was assessed in mice, following the experimental protocol represented in Fig 1.

STZ-D mice displayed mean levels of blood glucose >200 mg/dl through the experiment (Fig 3A). At the end of follow-up no significant differences in tumor volume were observed between control and STZ-D mice (Fig 3B and 3C).

Table 1. Clinicopathological characteristics of non-diabetic and diabetic colon cancer patients. Data expressed as mean ± SD or N (%).

Variable	*Overall (N = 160)*	*Non-diabetic (N = 79)*	*Diabetic (N = 81)*	P
Age years,	74.8 ± 10.4	72.9 ± 11.3	76.7 ± 9.2	0.02
Glucose levels, mg/dl,	112.4 ± 38.5	93.2 ± 9.0	131.1 ± 46.4	<0.0001*
Triglycerides, mg/dl,	117.8 ±71.0	95.4 ±36.1	138.0 ± 87.3	<0.0001*
Cholesterol, mg/dl,	165.8 ± 44.2	180.3 ±38.9	151.1 ±40.3	<0.0001*
BMI, kg/m^2,	24.4 ±2.8	23.5 ± 2.7	25.9 ±2.5	0.06
Lymphocytes, x10^3 μl,	2.1 ±0. 9	2.1 ± 0.8	2.1 ± 0.9	0.70
Neutrophils, x10^3 μl,	4.7 ± 2.1	4.3 ± 1.9	5.0 ± 2.1	0.02*
Platelets, x10^3 μl,	283.0 ± 116.0	279.2 ± 111.8	286.1 ± 120.1	0.70
Females, N (%)	67 (41.9%)	37 (46.8%)	30 (37%)	0.21
Clinical debut colon cancer, N (%)				
Subclinical	146 (91.2%)	74 (93.7%)	72 (88.9%)	0.28
Acute	14 (8.8%)	5 (6.3%)	9 (11.1%)	
ECOG, N (%)				
0	99 (61.9%)	56 (70.9%)	43 (53.1%)	0.02*
$\geq$ 1	61 (38.1%)	23 (29.1%)	38 (46.9%)	
CEA (ng/mL), N (%)				
$\leq$5	87 (54.4%)	47 (82.5%)	40 (75.5%)	0.39
>5	23 (14.4%)	10 (17.5%)	13 (24.5%)	
N/A	50 (31.2%)	-	-	
pT, N (%)				
T1-T2	53 (33.1%)	24 (30.4%)	29 (35.8%)	0.61
T3-T4	107 (66.9%)	55 (69.6%)	52 (64.2%)	
pN, N (%)				
N0	101 (63.1%)	48 (60.8%)	53 (65.4%)	0.54
N+	59 (36.9%)	31 (39.2%)	28 (34.6%)	
Grade, N (%)				
Low grade	147 (91.9%)	72 (91.1%)	75 (92.6%)	0.73
High grade	13 (8.1%)	7 (8.9%)	6 (7.4%)	
Adjuvant therapy, N (%)	54 (33.8%)	29 (36.7%)	25 (30.9%)	0.43
Tumor site, N (%)				
Right	75 (46.9%)	32 (40.5%)	43 (53.1%)	0.11
Left	85 (53.1%)	47 (59.5%)	38 (46.9%)	
Lymphovascular invasion, N (%)				
Yes	23 (14.4%)	12 (18.8%)	11 (16.4%)	0.72
No	108 (67.5%)	52 (81.2%)	56 (83.6%)	
N/A	29 (18.1%)	-	-	
Stage, N (%)				
Low	100 (62.5%)	49 (62.0%)	51 (63.0%)	0.90
High	60 (37.5%)	30 (38.0%)	30 (37.0%)	
Recurrence, N (%)	16 (10.0%)	7 (8.9%)	9 (11.1%)	0.63
Death, N (%)	23 (14.4%)	11 (13.9%)	12 (14.8%)	0.87
Cancer-related death, N (%**)	12 (52.1%)	5 (45.4%)	7 (58.3%)	0.53

* p<0.05 denotes statistical significance.

** % of total deaths.

Abbreviations: BMI, Body mass index; ECOG, Eastern Cooperative Oncology Group; CEA, carcinoembrionary antigen; SD, standard deviation; N/A, Not available

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Table 2. Cancer-related clinicopathological characteristics and metformin use in diabetic patients with colon cancer.

Variable	*Non Metformin (N = 46)*	*Metformin(N = 35)*	*P*
***ECOG**, N(%)*			
0	27 (58.7%)	16 (45.7%)	0.24
≥ 1	19 (41.3%)	19 (54.3%)	
***pT**, N(%)*			
T1-T2	18 (39.1%)	11 (31.4%)	0.47
T3-T4	28 (60.8%)	24 (68.5%)	
***pN**, N(%)*			
N0	31 (67.4%)	22 (62.9%)	0.67
N+	15 (32.6%)	13 (37.1%)	
***Grade**, N(%)*			
Low grade	42 (91.3%)	33 (94.3%)	0.69
High grade	4 (8.7%)	2 (5.7%)	
***Lymphovascular invasion**, N(%)*			
Yes	7 (15.2%)	4 (11.4%)	0.75
No	32 (69.6%)	24 (68.6%)	
N/A	7(15.2%)	7 (20%)	
***Stage**, N(%)*			
Low	30 (65.2%)	21 (60.0%)	0.63
High	16 (34.8%)	14 (40.0%)	
***Recurrence**, N(%)*			
Yes	6 (13.0%)	3 (8.6%)	0.75
No	40 (87.0%)	32 (91.4%)	
***Death**, N(%)*	7 (15.2%)	5 (14.3%)	0.90
***Cancer-related death**, N(%*)*	4 (57.1%)	3 (60%)	0.29

* % of total deaths

Abbreviations: ECOG, Eastern Cooperative Oncology Group; N/A, Not available

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The morphological analysis of tumors in control and STZ-D groups revealed identical architectural and cytologic characteristics, showing a homogeneous solid growth pattern with scattered differentiated glands, invasive tumor edge on surrounding soft tissues and focal intratumoral necrosis. Similarly, no differences in proliferation rates were observed (Fig 4A and 4B). The examination of other organs did not demonstrated dissemination of tumor cells.

Analysis of microvascular density in tumors using CD31 as endothelial marker for quantification also demonstrated similar results between control and STZ-D mice (Fig 4C).

Discussion

There are many epidemiological studies addressing the risk of developing cancer in diabetic patients, but few reports have focused on the influence of diabetes on colon cancer behavior once colon cancer has already developed. We now report that no differences in tumor behavior or characteristics were found when colon cancer in diabetic patients was compared with colon cancer in non-diabetic patients or when colon cancer xenografts were implanted in diabetic or control mice.

The main strength of the present study is the homogeneous, well-balanced and well-characterized population of the clinical study that limits the bias that may result from more heterogeneous or less characterized populations [12, 14, 27, 28]. Another strength is a preclinical study that supports the conclusions of the clinical dataset.

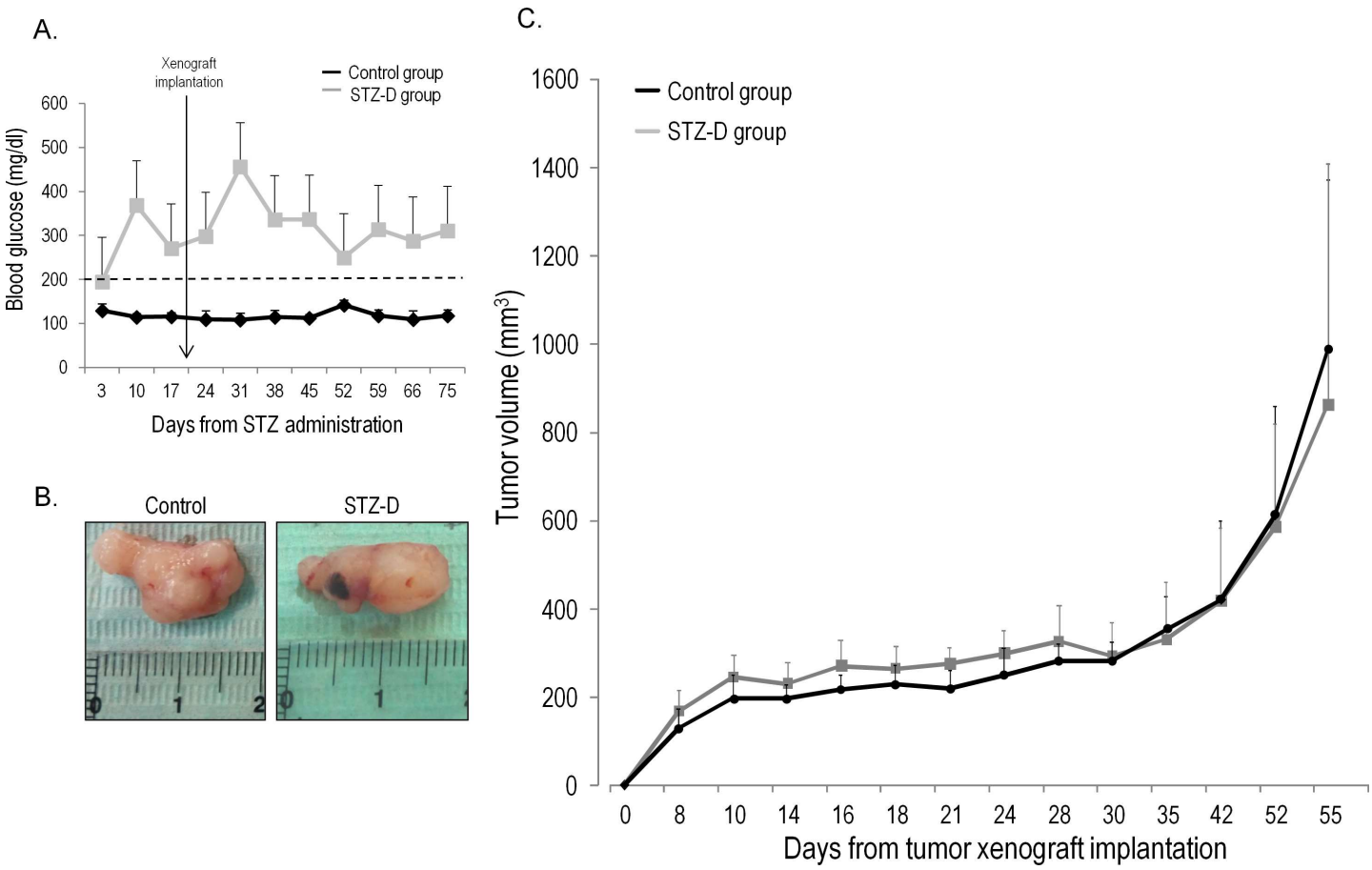

Fig 3. A diabetic environment does not modify tumor xenograft growth in mice. A) Blood glucose levels in diabetic and control mice during the experiment. The cutoff point to define development of diabetes was established at 200 mg/dl; **B)** Representative images of tumors from diabetic and control mice; **C)** Tumor growth curves for xenografts in diabetic and control mice.

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Our clinical results are in accordance to those in the "European American" ethnic group, ethnically closer to our cohort, in a multiethnic cohort [12]. Thus, there were no differences between diabetics and controls in terms of anatomical or pathological characteristics as stage or right/left localization. A previous report describing an association between diabetes and worse histopathologic colorectal cancer features may have been limited by excessive patient heterogeneity related to the inclusion of diverse histological types and of patients with rectal cancer [28]. Colon and rectal cancer show multiple biological and clinical differences, probably related to different mechanism of oncogenesis [24, 29, 30]. Furthermore, the standard treatment for rectal cancer shows more inter-institutional variability depending on the surgeon expertise. We excluded rectal cancer from our study in order to better preserve the anatomical structure. Standard rectal cancer treatment includes neoadjuvant chemo-radiation, which essentially modifies cell integrity and the microenvironment and may make the tumor disappear. Thus, rectal cancer patients merit a separate analysis. Adenocarcinoma is the most common colon cancer subtype and the most probably linked with a pro-inflammatory environment. Thus, we excluded other histologic types, since additional oncogenic mechanisms may contribute.

It has been suggested that medication used to control the glucose levels, mainly metformin, may modify the risk of developing cancer [31–34]. For this reason, we analyzed the metformin-treated subgroup in our cohort. We did not observe an effect of metformin on tumor

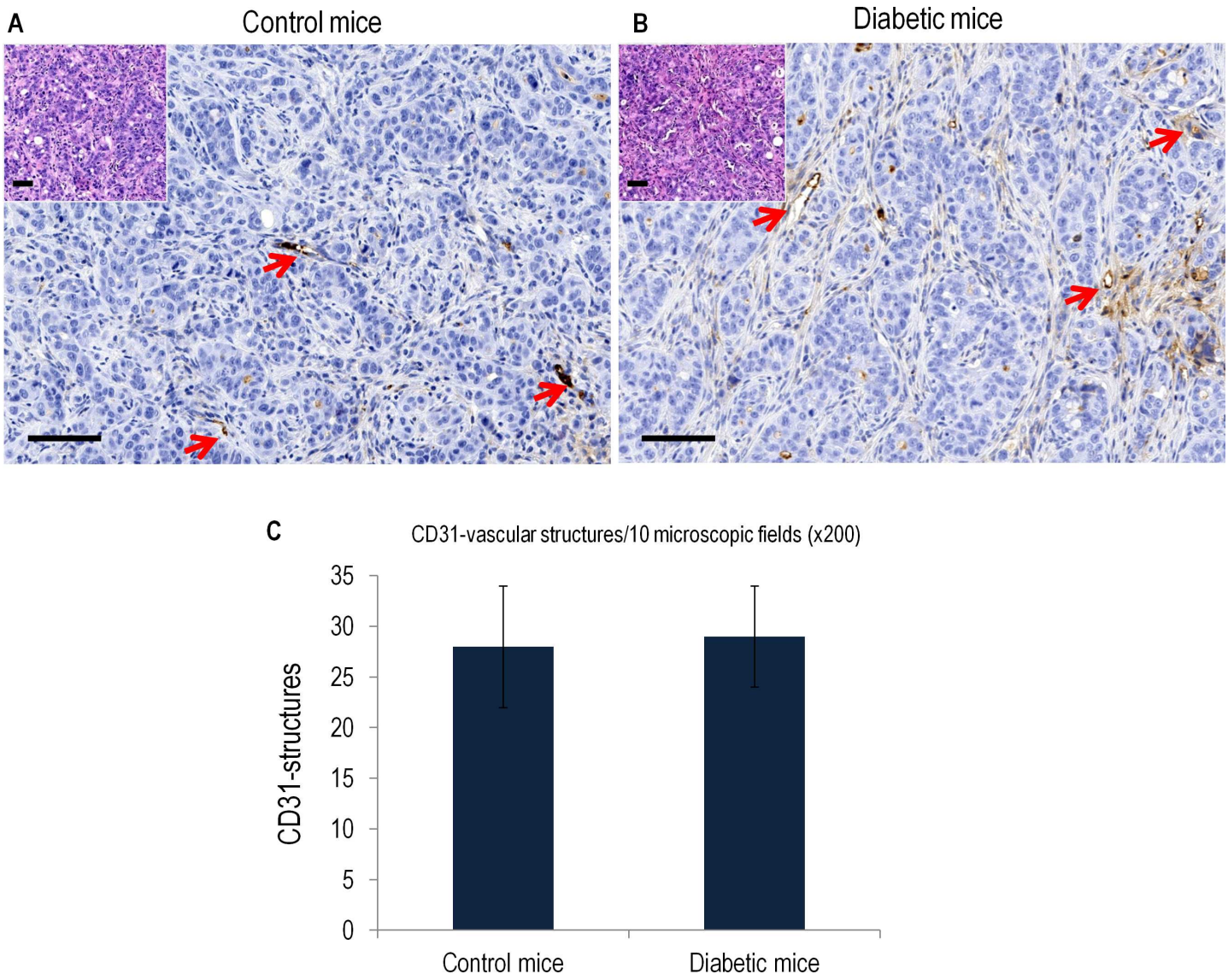

Fig 4. Representative histological images and corresponding quantification of vascular structures in the tumor xenograft model in mice. A) and B) show hematoxylin-eosin staining and CD31 immunohistochemistry images in tumor samples from control and diabetic xenografts. Original magnification x200. Arrows indicate vascular structures. **C)** Graphical representation of the number of vascular structures/10 microscopic x200 fields in control and diabetic mice. Scale, bar, 100 μm.

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behavior. However, we are very cautious about this result, since the additional stratification of data resulted in very low number of cases. Nevertheless, despite the few cases analyzed, results were concordant with several reports, which showed that metformin may reduce cancer risk, but its effect on mortality following cancer remains unclear [35, 36], even in other tumor types [37].

The preclinical *in vivo* model is based on published and widely accepted methods [38], yielding an homogeneous and reproducible experimental approach which allows for non-biased robust results. This experimental procedure disclosed no significant differences in tumor growth kinetics or histopathological characteristics between diabetic and control mice. Very few *in vivo* studies have addressed the influence of diabetes on the growth of human

cancer. One report did not observe significant differences in sarcoma xenograft growth kinetics between diabetic and control mice [23]. By contrast, in a similar experimental setting, a protective effect of diabetes in a prostate cancer xenograft model was reported [22]. However, the published patient cohort reports are heterogeneous regarding to the effect of diabetes on prostate cancer patients [39].

Among the weaknesses, we should mention the retrospective nature of the study and the relatively small study population, belonging to a single center. While the smaller size allowed a degree of detail in the analysis that is not possible in large epidemiological studies, the single center nature limits the extrapolation of results to other centers or countries.

To summarize, our findings point towards a mild or negligible influence of diabetes on colon cancer behavior once cancer has already developed. These results serve as the basis to design a larger, multicenter, confirmatory clinical study.

Supporting information

S1 Fig. Schematic representation of the selection of patients who met the inclusion criteria for database development.
(TIF)

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